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# SUBJECT INDEX

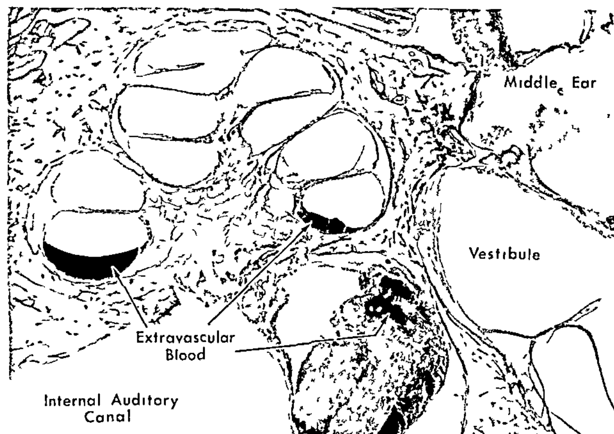



*Fig 1* View from the right ear of a 44-year-old male who died 2 weeks after experiencing a spontaneous subarachnoid hemorrhage Extravascular blood is seen in the internal auditory canal and in the scala ty pani of the basal turns of both ears The cochl aqueducts of both ears are patent and contain blood

Most of the ears in which the cochlear aqueducts were blocked he found displacement of Reissner's membrane toward the scalae vestibuli He also reported finding a glassy pre cipitate in the scalae tympani in these ears He reasoned that the displacement of Reiss ner's membrane toward the scala vestibuli was due to a decrease in the volume of peri lymphatic fluid and a compensatory increase in endolymphatic fluid to take up the space He concluded on this basis that perilymphatic fluid was derived from cerebrospinal fluid which passed through the cochlear aqueduct Subsequently, Nakamura (1967) repeated this experiment with similar results

Several other experiments, however, in which bone dust and bone wax was used to block the mid part of the duct, tend to refute the idea that blockage of the cochlear aque duct causes endolymphatic hydrops Linds et al (1952) blocked the cochlear aquedu of two sound conditioned cats and found morphological changes in the inner ears alterations in auditory acuity after surviv times of 4 and 8 months Schuknecht & K mura (1953) blocked both the cochlear aqu ducts and vestibular aqueducts in 2 cats ai after short survival times found mild end lymphatic hydrops In these animals it cou not be determined, of course, whether tl endolymphatic hydrops was the result of block age of the cochlear aqueduct or of the ve tibular aqueduct or both Schuknecht & Se (1963) blocked the cochlear aqueducts of cats and after survival times of 10 month found all to have normal membranous laby rinths on histological study

In view of the discrepancies in the resul

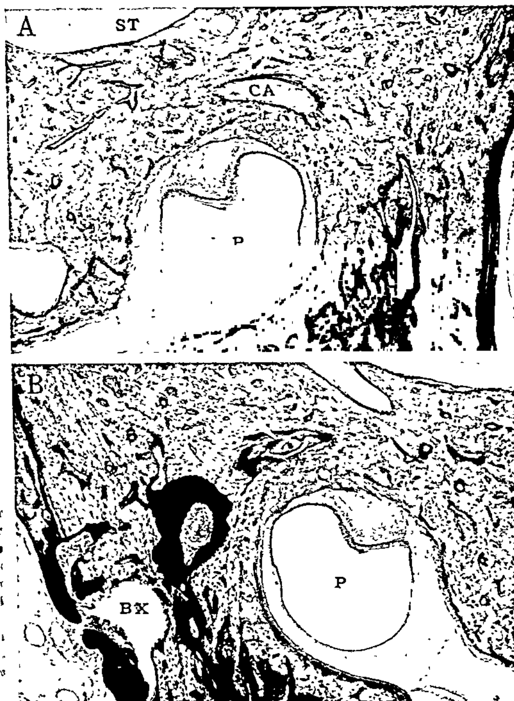



*Fig 2* (A) Photomicrograph showing the normal cochlear aqueduct (*CA*) situated between the posterior ampulla (*P*) and the scala tympani (*ST*) Guinea pig (B) This photomicrograph shows the obliterated cochlear aqueduct (*arrow*) with new bone formation The clear space (*BX*) represents the drilled area filled with bone wax which has been dissolved Survival time, 5 months. *P*, posterior ampulla.

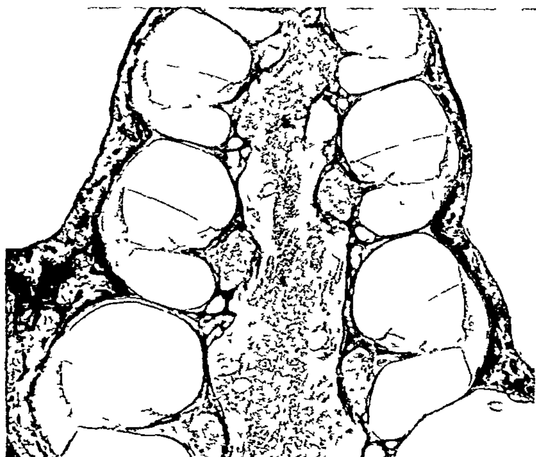


*Fig 3* Photomicrograph of cochlea in which the cochlear aqueduct is obliterated Note the normal position of Reissner s membrane and the normal organ of Corti spiral ganglia and stria vascularis Survival time 5 months. The same specimen is shown in Fig 2 B Guinea pig

of these studies we repeated the experiment using guinea pigs. We used guinea pigs because of their proclivity for developing endolymphatic hydrops as evidenced in experiments on obstruction of the vestibular aqueduct The cochlear aqueduct of the guinea pig is easy to obstruct because it is comparatively long narrows at its mid point and pursues a course separate from the inferior cochlear vein In one group of animals we blocked the cochlear aqueducts only and in a second group we blocked both the cochlear and vestibular aqueducts at the same time We reasoned that if the source of perilymph is interrupted by blocking the cochlear aqueduct the extent of hydrops resulting from the obstruction of the vestibular aqueduct should be enhanced

## MATERIALS AND METHODS

A total of 30 guinea pigs weighing 270 to 400 grams were used for this study In the first group of 25 animals in which only the cochlear aqueducts were obliterated 21 were processed for light microscopy and 4 for electron microscopy In each animal both ears were prepared for study, with one serving as the experimental ear and the other as a control The specimens for electron microscopy (survival time 2–8 months) were fixed with 1% phosphate buffered osmium, embedded in Epon cut with an LKB ultrotome stained with uranyl acetate and lead citrate and examined with a Siemens Elmiskop 1 The specimens for light microscopy (survival time 1–3 months 5 animals, 4–7 months 16 animals)

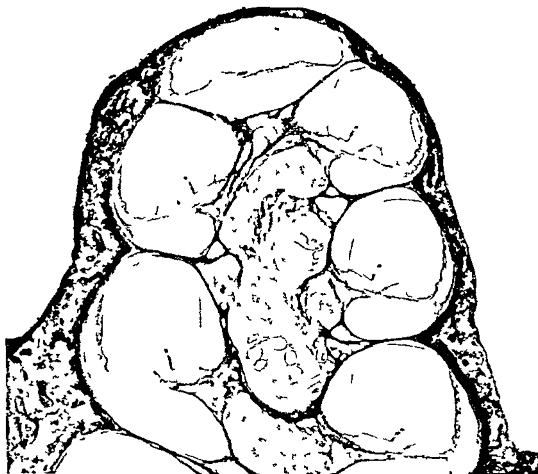



*Fig 4* (A) Normal endolymphatic duct and sac (*ES*) adjacent to the common crus (*C*) *S* Sigmoid sinus Guinea pig (B) Obliterated vestibular aqueduct (*arrow*) Note the endolymphatic sac (*ES*) with the increased rugose arrangement distal to the interrupted area *S* Sigmoid sinus *C* Common crus Survival time 5 months

were perfused with Heidenhain Susa embedded in celloidin and cut serially at 20 $\mu$m thickness

In the second group of 5 animals both the cochlear and vestibular aqueducts were obliterated during the same surgical procedure All of these animals were processed for light microscopic study (survival time 2–5 months)

The surgical method for blockage of the cochlear aqueduct was similar to the procedure we (Kimura & Schuknecht 1965) previously used for blockage of the vestibular aqueduct After medial retraction of the cerebellum, the cranial aperture of the cochlear aqueduct was visualized A 4/0 cutting burr was used to drill into the cranial aperture of the aqueduct and follow it for a distance of about 1 5 mm at which point the lumen was very small The lumen of the aqueduct was then packed with bone dust and covered with bone wax The vestibular aqueduct was similarly blocked about 2 mm from the margin of the



5 Photomicrograph of cochlea in which both cochlear and vestibular aqueducts are obliterated an extensive hydrops and atrophy of the neurosensory elements and the stria vascular s in the apica turns The same specimen is shown in F g 4 B Survival time 5 months Guinea p g

operculum The opening on the skull dura was covered with gelfoam and the overlying soft tissues were closed in two layers

## FINDINGS

The cochlear aqueduct was successfully blocked in all ears prepared for light microscopic study (Fig 2) however four had to be eliminated from the study for the following reasons two inadvertent arterial obstruction one inadvertent venous obstruction and one bilateral (presumably pre-existing) atrophy of the organs of Corti

None of the seventeen successfully operated ears showed endolymphatic hydrops (Fig 3) A slight displacement of Reissner s membrane into the scala vestibuli in the basa turns near the internal aperture of the cochlear aqueduct was noted in three specimens however the opposite control ears showed this same change All sensory cells including the organ of Corti maculae utriculi and sacculi and cristae appeared normal The other membranous structures including the stria vascularis spiral ligament, limbus spiralis and tectorial membrane also appeared normal The endolymphatic fluid appeared clear The perilymphatic spaces of most experimental ears contained a fine granular precipitate which was present in only a few of the control ears

The four ears prepared for electron microscopy were not evaluated as to the success of blockage though the cranial aperture was

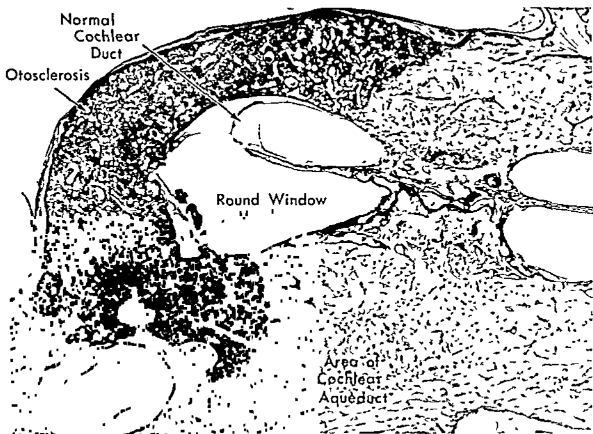



*Fig 6* Photomicrograph of left temporal bone in a 69 year-old male with bilateral otosclerosis Both ears show extensive otosclerotic involvement of the lateral parts of the bony labyrinths with obliteration of oval and round windows and cochlear aqueducts There was no evidence of endolymphatic hydrops in either ear

covered by new bone One of the specimens showed a bilateral atrophy of the outer sensory cells The only change noted in other ears was the presence of vacuoles in the efferent nerve endings to the outer hair cells, but this change was also noted in the control ears The afferent nerve endings showed no morphological change, nor did the subcuticular zones of the sensory cells The stria vascularis appeared normal in all ears

The experimental ears of the 5 animals in which both the cochlear and vestibular aqueducts were obliterated, exhibited endolymphatic hydrops involving the cochlear duct, saccule and utricle (Figs 4 and 5) In addition, there were degenerative changes in the membranous labyrinths of four of the five ears The ear of one animal (survival time 2 months) showed a reduction in spiral ganglia in the fourth turn A second animal (survival time 2 months) exhibited atrophy of the spiral ganglia and outer hair cells in the third and fourth turns A third (survival time 5 months) presented loss of spiral ganglia and the outer sensory cells in the first, third and fourth turns The stria vascularis was cystic and atrophic in a limited area of the first and fourth turns of this animal In the experimental ear of the fourth animal (survival time 5 months) there was a slight decrease in population of the spiral ganglia in the first and fourth turns, and of the outer hair cells in the second and fourth turns In this ear the stria and spiral ligament were totally mis-

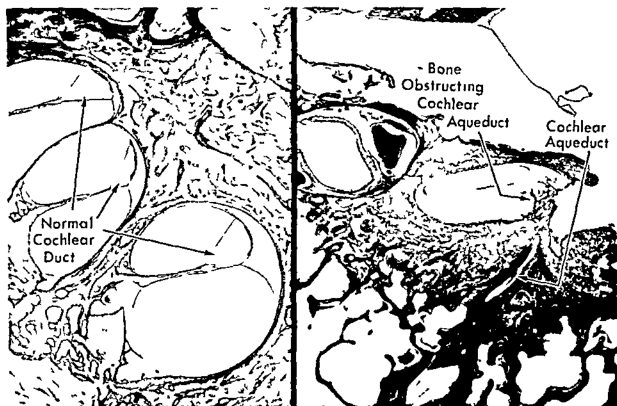



*Fig 7* Photomicrograph from left ear of a 50-year old male who was profoundly deaf in both ears from early life There is bone in the scala tympani of the basal turn which is obstructing the opening of the cochlear aqueduct There is atrophy of the organ of Corti throughout but the stria vascularis appears normal and there is no evidence of hydrops

g in a small region of the basal turn A few polymorphonuclear leucocytes were found in the perilymphatic space in this ear suggesting the presence of a mild inflammatory reaction In general, these changes are of similar type and magnitude to those seen in previous experiments in guinea pigs in which only the vestibular aqueduct was obliterated (Kimura, 1967)

Light microscopic studies of the surgically obliterated cochlear aqueducts showed the lumens to be plugged by a thin layer of fibrous tissue upon which was superimposed a clear zone representing the area occupied by bone wax which was dissolved during the histological preparation, and finally by an irregular layer of new bone In the gross specimens the bone wax appeared hardened when manipulated with a dissecting needle It seemed to remain intact and surrounded by new bone even after postoperative survival times of 5 months There was some increase in fibrous tissue within the aqueducts, however, the tympanic orifices appeared identical to those of the control ears

Examination of the obliterated vestibular aqueducts revealed a similar pattern of pathological change, that is fibrous tissue plugs at the cranial ends of the ducts with overlying layers of bone wax and irregular new bone

Most of the animals of both groups showed no dysequilibrium or nystagmus following surgery Slight nystagmus lasting for one day following surgery was noted in a few animals

## DISCUSSION

We believe the results of this experiment show conclusively that blockage of the cochlear aqueduct of the guinea pig causes no signifi

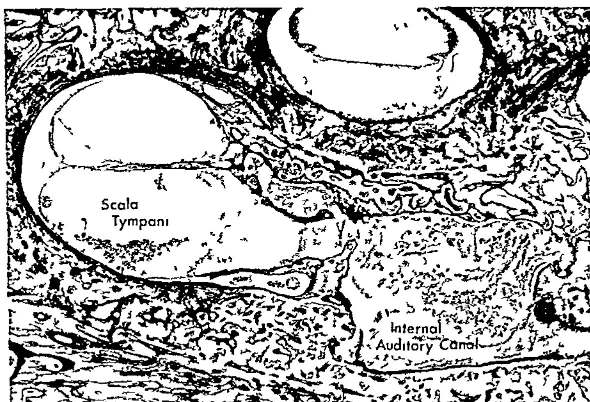



*Fig 8* Photomicrograph from right ear of a 17 month-old male who died of acute pneumococcal meningitis Pus cells are seen in the internal auditory canal and in the perilymphatic spaces of both ears In the right ear there is a direct communication between the internal auditory canal and the scala tympani of the basal turn.

cant morphological change in the inner ear This observation is in agreement with the results of similar experiments by Lindsay et al (1952) and Schuknecht & Seifi (1963) who used cats and is in contradiction with the findings of Uyama (1933) who used rabbits and Nakamura (1967) who used guinea pigs Although it is pure speculation we think it is possible that the hydrops produced by Uyama and Nakamura was of inflammatory etiology and caused either by surgical trauma or as a reaction to the sarcoma cells or the catgut suture

The findings in the five ears in which both the cochlear aqueducts and vestibular aqueducts were blocked were no different from those we have observed previously from blockage of the vestibular aqueducts alone when postoperative survival times were the same Thus it appears that the endolymphatic hydrops and the atrophy of sensory and neural structures in these animals were the result of obstruction of the vestibular aqueducts and the consequent loss of function of the endolymphatic sacs and furthermore that these changes were not enhanced by blockage of the cochlear aqueduct

Observations on histologically prepared human temporal bones further confirms the findings of our animal experiment In our collection there are several specimens which show fibrous or osseous obliteration of the cochlear aqueducts without evidence of endolymphatic hydrops or other significant morphological change (Figs 6 and 7) In contradistinction to animals the cochlear aqueduct is only rarely widely patent in man (Schuknecht 1971) and usually it is very narrow This ob-




*Fig 9* Right ear of a 65 year-old woman who died 24 ours after a spontaneous subarachnoid hemorrhage ere is a massive infiltrate of fresh extravascular around the nerve trunks in the internal audi canal The blood cells have extended along the nerve bundles to enter Rosenthal s canal and the fluid channels of the modiolus The cochlear aqu duct is extremely small and no blood is seen beyo its mid portion, nor is there blood in the inner ear

servation, also made by others (Waltner, 1948, Ritter & Lawrence, 1965) has already posed the question as to the functional importance of this structure

Because of the chemical similarity of CSF and perilymph it seems to us rather probable that perilymph derives from the CSF and, possibly, *from the fenestrated capillaries found* among the modiolar cells (Kimura & Ota, 1973) It is obvious that perilymph is maintained in the presence of a blocked cochlear aqueduct Our experimental results lead us to look for other routes by which the CSF may reach the inner ear The most probable would seem to be via channels from the internal auditory canal and there are two routes by which this might occur (1) There can be a direct communication from the internal aud tory canal to the scala tympani of the bas turn This route has been clearly observe in human ears and is the route by which in fection may spread from the meninges to th inner ear (Schuknecht & Montandon, 1970) (See Fig 8) (2) In some human ears ther is a *communication from the subarachnoi* space of the internal auditory canal to th spaces located in the modiolus Schuknech & Holden (1968) have observed that in som individuals these communications are larg enough to accommodate erythrocytes (Fig 9) How CSF might reach the perilymphatic scala from these modiolar spaces is not clear The most probable route would seem to be alon the perineural spaces of the auditory and ves-

tibular nerve fibers In the cochlea the CSF could then pass from the perineural spaces of the osseous spiral lamina through the canaliculae perforantes (Schuknecht et al, 1959, Lim, 1970, Tanaka et al, 1973) to reach the scala tympani

Another hypothesis is that perilymph is a blood filtrate arising directly from the vessels located in the spiral ligament and elsewhere (Altmann & Waltner, 1950, Kley, 1951, Hawkins, 1967) Several studies have shown that the spiral ligament is anatomically related to the perilymphatic space and has many channels communicating with it (Takahashi & Kimura, 1970)

## ZUSAMMENFASSUNG

Es wurde versucht, beim Meerschweinchen nachzuweisen ob der Verschluss des Aquaeductus cochleae morphologische Veranderungen im Innenohre bewirken kann, und insbesondere ob es moglich ist, endolymphatischen Hydrops zu erzeugen Bei siebzehn Tieren, bei welchen der Aquaeductus cochleae mit Erfolg und ohne Beschadigung anderer Strukturen verschlossen wurde, waren bei der lichtmikroskopischen Untersuchung nach postoperativen Überlebenszeiten von ein bis sieben Monaten keine histologischen Veranderungen zu finden Auch durch Untersuchungen am Elektronenmikroskop liessen sich keine feinen Strukturveranderungen nachweisen Bei funf Versuchstieren mit postoperativen Überlebenszeiten von zwei bis funf Monaten bei welchen sowohl der Aquaeductus cochleae als auch der Aquaeductus vestibuli verschlossen wurden, fanden wir endolymphatischen Hydrops und Degeneration der sensoriellen und neuralen Strukturen von anatomisch gleichem Aspekt und Ausmass wie bei vorhergehenden Versuchen, bei welchen nur der Aquaeductus vestibuli blockiert wurde Es geht daraus hervor, dass die Funktion des Aquaeductus cochleae, der bei den meisten Saugetieren und manchmal beim Menschen eine weite Verbindung zwischen dem Liquor cerebrospinalis und der Perilymphe darstellt, ohne schadliche Auswirkungen auf das normale Innenohr ausgeschaltet werden kann Andere mogliche Ursprunge der Perilymphe sind die Liquorflussigkeit des Subarachnoiden Raums des inneren Gehorgangs und ein Filtrat aus den Blutgefassen des Innenohres

## REFERENCES

Altmann, F & Waltner J G 1950 Further investigations on the physiology of the labyrinthine fluids *Ann Otol 59* 657

Beentjes, B I J 1972 The cochlear aqueduct and the pressure of cerebrospinal and endolabyrinthine fluids *Acta Otolaryng* (Stockh ) *73*, 112

Hawkins, J E, Jr 1967 Vascular patterns of the membranous labyrinth In *Third symposium on the role of the vestibular labyrinth in space exploration*, p 241 NASA SP 152 National Aeronautics and Space Agency, Washington, D C

Kimura, R S 1967 Experimental blockage of the endolymphatic duct and sac and its effect on the inner ear of the guinea pig *Ann Otol 76* 664

Kimura, R S & Ota, C Y 1973 The ultrastructure of the cochlear blood vessels *Acta Otolaryng* (Stockh ), in press

Kimura, R S & Schuknecht, H F 1965 Membranous hydrops in the inner ear of the guinea pig after obliteration of the endolymphatic sac *Pract Oto-rhinolaryng* (Basel) *27*, 343

Kley, E 1951 Zur Herkunft der Perilymphe (Ein Beitrag zur Physiologie des Innenohre) *Z Laryng Rhinol Otol 30*, 486

Lim, D J 1970 Surface ultrastructure of the cochlear perilymphatic space *J Laryng 84* 413

Lindsay, J R, Schuknecht, H F, Neff, W D & Kimura, R S 1952 Obliteration of the endolymphatic sac and the cochlear aqueduct *Ann Otol 61* 697

Nakamura, T 1967 Experimental obliteration of the endolymphatic sac and the perilymphatic duct (Histological and biochemical studies) *Jap J Otol Tokyo 70* 932

Perlman H B & Lindsay, J R 1939 Relation of the internal ear spaces to the meninges *Arch Otolaryng* (Chic ) *29* 12

Ritter, F N & Lawrence, M 1965 A histological and experimental study of cochlear aqueduct patency in the adult human *Laryngoscope 75* 1224

Schuknecht, H F 1971 *Stapedectomy* Little, Brown & Company, Boston 119 pages

Schuknecht H F, Churchill J A & Doran R 1959 The localization of acetylcholinesterase in the cochlea *Arch Otolaryng* (Chic ) *69* 549

Schuknecht, H F & Holden H B 1968 Distribution of blood in the inner ear following spontaneous subarachnoid hemorrhage *J Laryng 82* 321

Schuknecht, H F & Kimura, R S 1953 Functional and histological findings after obliteration of the periotic duct and endolymphatic sac in sound conditioned cats *Laryngoscope 63* 1170

Schuknecht H F & Montandon, P 1970 Pathology of the ear in pneumococcal meningitis *Arch Klin Exp Ohr Nas Kehlkopfheilk 195* 207

Schuknecht H F & Seifi, A E 1963 Experimental observations on the fluid physiology of the inner ear *Ann Otol 72* 687

Takahashi, T & Kimura, R S 1970 The ultrastructure of the spiral ligament in the Rhesus monkey *Acta Otolaryng* (Stockh ) *69* 46

Tanaka, T, Kosaka, N, Takiguchi, T Aoki, T & Takahara, S 1973 Observation on the cochlea with SEM In *Scanning Electron Microscopy* Part III, p 427

Uyama, Y 1933 Histopathologische Veranderungen

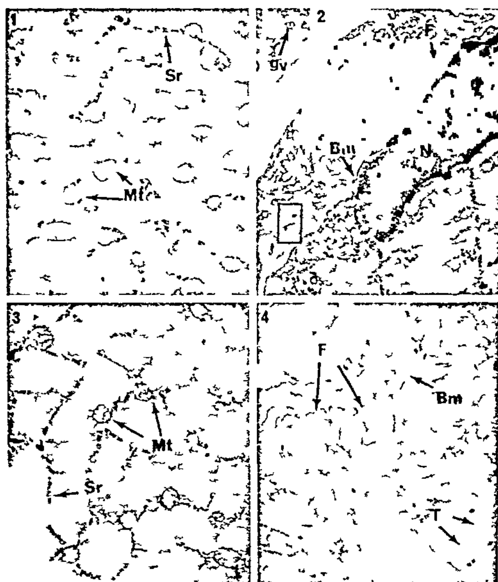



*Fig 1* Transverse section of slow fiber showing poorly defined myofibrils little sarcoplasmic reticulum (*Sr*) and numerous mitochondria (*Mt*) × 15 000

*Fig 2* Nerve ending of slow fiber A granule containing vesicle (enclosure) is shown at higher magnification in the inset (*Gv*) The junctional foldings (*F*) are very slight *Bm* Basement membrane N Nucleus × 10 500 × ?1 000

*Fig 3* First type of twitch fiber in transverse section Myofibrils are well delineated by sarcoplasmic reticulum (*Sr*) The mitochondria (*Mt*) are small and sparse × 15 000

*Fig 4* Nerve ending of first type of twitch fiber in longitudinal section showing well developed junctional foldings (*F*) on a raised sole area *T* Transverse tubules *Bm* Basement membrane × 10 500

across the myofibril an M line is clearly seen The nerve endings of such fibers (Fig 4) have a large elevated sole area and well developed junctional foldings

The second type of twitch fiber in transverse (Fig 6) and longitudinal (Fig 7) section displays myofibrils delineated by sarcoplasmic reticulum moderate amounts of glycogen and numerous mitochondria which tend to aggregate and form chains Compared to the first type of twitch fiber the transverse tubular system appears less elaborate the Z line is somewhat wider the M line is less clearly discernible and the junctional foldings at the nerve ending (Fig 7) appears less well developed

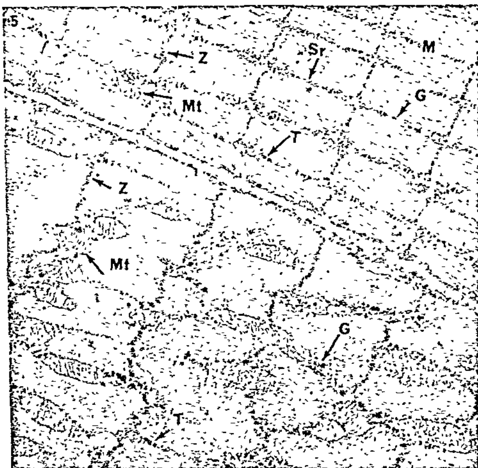



*Fig 5.* Longitudinal section of slow fiber (*bottom*) and first type of twitch fiber (*top*). The myofibrils of the slow fiber are not well delineated, while those of the twitch fiber are clearly separated The Z line is wider in the slow fiber compared with that of the twitch fiber. *Mt*, mitochondria; *Sr*, Sarcoplasmic reticulum; *T*, transverse tubules; *G*, Glycogen particles; *M*, M line × 15 000.

Laminated structures were observed under the cell membrane of a twitch fiber (Fig. 8). The dark lines show a periodicity of 1 430–1 860 Å Multivesicular bodies appear associated with this laminated structure. The adjacent cellular space is devoid of myofibrils, but contains granular material, much glycogen, and many mitochondria some of which appear vacuolated, or broken

## DISCUSSION

The morphologically slow fiber observed in this study is essentially comparable to those reported in other middle ear muscles (Erulkar et al., 1964; Fernand & Hess, 1969; Hirayama & Daly, in preparation; Seiden, 1971). The mitochondrial distribution of this fiber, in terms of their considerable number and even dispersion, is similar to that of the slow fiber in tensor tympani of rabbit (Hirayama & Daly, in preparation) Reports on the middle ear muscles of other animals do not fully describe the mitochondrial distribution of the slow fibers (Erulker et al., 1964; Fernand & Hess, 1969, Seiden, 1971). The slow fibers of latissimus dorsi anterior muscle of chicken has numerous mitochondra (Hess, 1961) while slow fibers in extraocular muscle of rat have but few (Mayr, 1971). The wide Z line of this

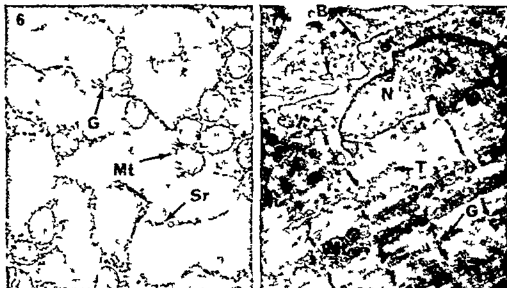






*Fig 6* Second type of twitch fiber in transverse section showing *myofibrils clearly separated by* sarcoplasmic reticulum (*Sr*) The numerous large mitochondria (*Mt*) tend to form clusters *G* Glycogen particles × 15 000

*Fig 7* Longitudinal section of second type of twitch fiber with nerve ending The mitochondria (*Mt*) appear in clusters and long chains The junctional foldings (*F*) are moderately developed *G* Glycogen particles *T* Transverse tubules *Bm* Basement membrane *N* Nucleus × 10 500

*Fig 8* Laminated structures (*L*) in twitch fiber associated with multivesicular bodies (*Mb*) The adjacent cell portion contains numerous granular clusters many glycogen granules and mitochondria some of which are vacuolated or broken (*double arrows*) This atypical portion of the cell also shows a deep invagination (*single arrow*) of the cell membrane × 15 000

slow fiber is consistent with reports of such fibers in amphibia (Page, 1965, Peachey & Huxley, 1962) and in rat extraocular muscle (Mayr, 1971) The presence of granule-containing vesicles in the terminal axon of this slow fiber agrees with descriptions of such fibers in extraocular muscle of cat (Cheng & Breinin, 1965)

The first and second types of twitch fibers and their associated nerve endings appear consistent, respectively, with descriptions of white and red types of fibers in mammalian skeletal muscle (Padykula & Gauthier, 1967) Previous reports of middle ear muscles described but one type of twitch fiber in each muscle (*Erulkar et al*, *1964 Fernand & Hess, 1969*, Hirayama & Daly, in preparation, Seiden, 1971)

The laminated structure is comparable to that reported in the sensory epithelium of the inner ear (Friedmann, 1967, Hilding et al, 1967, Jahnke, 1969), the brain of cat (Morales & Duncan, 1966, Smith et al, 1964) and rat (Nauman & Wolfe, 1963) and extraocular muscle of man (Mukuno, 1966) Some investigators consider this structure to be associated with pathological changes, insofar as it was observed in the inner ear of patients with Meniere's disease (Friedmann, 1967) and in experimental ototoxic (Jahnke, 1969) or congenitally deaf animals (Hilding et al, 1967) It has also been suggested that they may serve as an anchoring device in extraocular muscle (Mukuno, 1966) In the present case, this structure is associated with an unusual appearance of the subsarcolemmal region, apparently far removed from the fibrillar structures


## ACKNOWLEDGMENT

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## ZUSAMMENFASSUNG


Eine morphologisch „schwerfallige" Muskelfaser wurde beobachtet sowie zwei Arten von „Zupffasern" Die „schwerfallige Muskelfaser hatte kaum getrennt Myofibrillen wenig sarkoplasmatisches Retikulum wenige querlaufende Tubulen und mehrere winzige Mitochondrien Die beiden „Zupffasern sahen der weissen bzw der roten Skeletalmuskelfiber ahnlich Ein blattriges Gefuge wurde ebenfalls beobachtet


## REFERENCES


Adinolfi A M & Pappas G D 1968 The fine structure of the caudate nucleus of the cat *J Comp Neurol 133* 167

Cheng K & Breinin G M 1965 Fine structure of nerve endings in extraocular muscle *Arch Ophthal* (Chic) *74* 822

Erulkar, S D, Shelanski M L, Whitsel B L & Ogle P 1964 Studies of muscle fibers of the tensor tympani of the cat *Anat Rec 149* 279

Fernand V S V & Hess A 1969 The occurrence structure and innervation of slow and twitch muscle fibers in the tensor tympani and stapedius of the cat *J Physiol 200* 547

Friedmann I 1967 I Electronmicroscope studies of the diseased macula of the utricle of the human inner ear with particular reference to Menière s disease In *Submicroscopic structure of the inner ear* (ed S Iurato)

Hess A 1961 Structural differences of fast and slow extrafusal muscle fibers and their nerve endings in chickens *J Physiol 157* 221

Hilding D A Sugiura A & Naka Y 1967 Deaf white mink Electronmicroscopic study of the inner ear *Ann Otol 76* 647

Hirayama M & Daly J F 1973 Fine structure of tensor tympani muscle in the rabbit *Acta Otolaryng* (Stockh) in press

Jahnke V 1969 Ultrastructure of the vestibular sensory areas in experimental lathyrism *Acta Otolaryng* (Stockh) *68* 336

Mayr, R 1971 Structure and distribution of fiber types in the external eye muscles of the rat *Tissue and Cell 3* 433

Morales R & Duncan D 1966 Multilaminated bodies and other unusual configurations of endoplasmic reticulum in the cerebellum of the cat An electronmicroscopic study *J Ultrast Res 15* 480

Mukuno K 1966 The fine structures of the human extraocular muscles (1) A "laminated structure in the muscle fibers *J Electron Microscopy 15* 227

Nauman R A & Wolfe, D C 1963 A striated intercellular material in rat brain *Nature 198* 701

Padykula H A & Gauthier, G F 1967 The ultra structure of neuromuscular junctions of mammalian red and white skeletal muscle fibers *JCB 35* 155 A

Page S G 1965 Comparison of the fine structure of frog slow and twitch muscle fibers *JCB 26* 477

Peachey L D & Huxley A F 1962 Structural

identification of twitch and slow striated muscle fibers of the frog *JCB 13*, 177
Reynolds E. S 1963 The use of lead citrate at high pH as an electron-opaque stain in electron-microscopy *JCB 17*, 208
Seiden, D 1971 Slow muscle fibers in the tensor tympani muscle of the guinea pig *Amer J Anat 132* 267
Smith, J M, O Leary, J L., Harris, A B & Gay, A J 1964 Ultrastructural features of the lateral geniculate nucleus of the cat *J Comp Neurol 123*, 357

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# CHANGES IN AUDITORY LOCALIZATION DURING BODY TILT

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*Abstract* The induction of an error between a subject's true orientation and his registered orientation in relation to gravity results in auditory mislocalizations of a similar size and time course The presence of visual cues prevents the development of errors in the interpretation of posture and, accordingly, prevents the development of errors in auditory localization as well These observations are interpreted as evidence for a spatial reference system responsible for the maintenance of auditory and visual direction constancy They demonstrate that where a subject hears a sound is dependent not only on the auditory cues at his ears but also on his registered orientation in relation to the gravitational force vector

The constancy of auditory and visual direction is a familiar perceptual phenomenon When an observer moves in relation to a stationary auditory or visual stimulus object he perceives himself as moving and not the stimulus He knows also how much he has moved and where the stimulus object is with respect to his new position If his performance were based solely upon the sensory cues from the stimulus source he could not determine whether he or the source had moved In general, if the change in stimulus cues at the receptor surfaces represents a change of $\Delta x$ in relative observer–source articulation, then $\Delta x$ can result from movement of the observer by distance $x$ and by movement of the source $x-\Delta x$, or the converse, where $x$ may vary from zero to some finite distance dependent on the resolving characteristics of the receptor organs

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Consequently, in maintaining constancy of auditory and visual direction an observer must utilize, in addition to sensory cues, knowledge about the ongoing orientation of his eyes and ears For example, in pointing to an external sound source, he must take into account the auditory cues from the sound source and the position of his head with respect to his trunk and hand Since his ears are stationary in relation to his head, it is often thought that the observer can make judgments about the position of the sound source in relation to his head simply on the basis of the auditory cues without taking into account the orientation of his head

That an observer can localize a sound source is in itself presumptive evidence for the existence of an internal reference system that permits him to relate the auditory cues at his ears to the moment to moment spatial orientation of his head and body It is this neural reference system relating postural and sensory information that permits him to make two types of auditory judgments, head relative localizations (saying whether the sound is to the left or right of the median plane of the head) and more general body relative localizations

(pointing to the sound, saying whether it is to the left or right of the torso, or left knee, etc )

It seemed likely that if an error could be induced in the relating of the auditory cues as given at the ears to postural information about body orientation that errors in auditory localization would result *A central concern* of the experiments to be described was to demonstrate that when a postural error is introduced not only "pointing localizations" but all head relative localizations to an ambient sound are affected In other words it is being claimed that even when an observer is saying whether a sound source is to the left or right of the median plane of his head he is taking into account the perceived position of his head with respect to his trunk and with respect to the gravitational vertical, as well as the auditory cues from the sound source

To test the validity of this assumption it was necessary to find a way to introduce an error between the observer's true head position and his registered head position Several earlier experiments furnished a clue Teuber & Liebert (1956) had found that a tilted subject in setting an ambient sound source to his median ane consistently places the source too far to upward side, i e, he "underestimates' the position of his median plane By contrast, under identical tilt conditions, a subject asked to set a dichotically presented sound to his median plane hears the sound as centered only when his down ear is being given a considerable time advantage relative to his other ear (Teuber & Diamond 1956)

It had been noticed too that a blindfolded subject who has been tilted and then returned toward the upright considers himself as upright when he is still tilted somewhat in the original direction (Clark & Graybiel 1963, Fleishman 1953 Mann & Passey, 1951 Mann & Ray, 1956 Passey & Guedry 1949 Teuber & Mishkin 1954) This underestimation effect is a very strong one after being tilted for a brief period of time the subject actually feels less tilted than he is

The possibility arises that the underestimation of body tilt in blindfolded subjects is responsible for the auditory displacements observed by Teuber & Liebert (1956) and Teuber & Diamond (1956) If these factors are in fact related, then the magnitude of the auditory errors during body tilt should be directly *proportional to the magnitude of the* postural adaptations to tilt, and have similar time courses Accordingly, these two experimental paradigms, when combined, furnish a technique for testing the hypothesis that a discrepancy between a subject's true orientation and his perceived or 'registered body orientation leads to systematic errors in auditory direction

An additional prediction is possible When a blindfolded subject, after prolonged tilt is returned to the upright his auditory localizations should exhibit aftereffects as a consequence of his feeling tilted past the vertical somewhat to the opposite side How auditory localizations would be affected if the subject were tilted but not blindfolded is less readily predicted in this situation the subject has veridical visual information concerning his orientation relative to gravity as well as misinformation from either vestibular or somatic sensory sources or both In general, in situations involving a conflict between visual and postural cues the visual cues predominate (Hay et al, 1965, Lackner, 1970 Nielsen, 1963) consequently, it might be expected that the auditory localizations of a tilted subject permitted unrestricted vision would be veridical

The experiments to be described systematically explored these issues as well as the distinction made by Teuber & Liebert (1956) and Teuber & Diamond (1956) that the errors in localization of ambient and dichotic sounds during body tilt are in opposite directions

## SUBJECTS

Twenty four male M I T undergraduates were paid for their voluntary participation Prior to being recruited each candidate s auditory

thresholds were measured with a Bekesy audiometer, to qualify for inclusion, his pulsed tone audiograms for both ears had to be within 5 dB of each other throughout the frequency range 100–10 000 Hz. In addition, no one with tonal lacunae or known vestibular defects was included For every prospective subject meeting these requirements two failed

## APPARATUS

### *Tilt chair*

The tilt chair used in these experiments provided lateral tilts in three discrete steps, left or right, to a maximum of 28° When a subject was being moved toward or away from the upright, the experimenter could read the tilt angle from a scale to the nearest $^{1}/_{2}$° An adjustable head cushion was mounted on the back of the chair and a metal slide supporting a vertical, 60° coronal arc of 12 inch radius was attached to its frame A small carriage holding a single Grason-Stadler headphone that was used to deliver auditory stimuli during ambient sound localization tasks could be moved noiselessly on the metal arc The "zero" of the calibrated arc corresponded to the median plane of a seated subject s head The face of the earphone was mounted in the slide carriage perpendicular to the base of the chair, a pointer permitted readings of its position to the nearest $^{1}/_{4}$° An adjustable head holder and chin rest held the subject's head rigid during experimentation

### *Sound generating equipment*

Auditory clicks were generated by gating a white noise source for 1 msec, independent control of the duration and intensity of signals in two channels was possible For dichotic presentations the interchannel separation could be varied continuously from simultaneity to 1 sec with either channel leading For ambient presentations only one channel was used A detailed description of the auditory apparatus is available in Lackner (1973)

## AUDITORY PROCEDURES

### *Dichotic localization*

Before auditory localization measurements were made, click detection thresholds were determined for each of a subject's ears so that the stimuli to be localized could be set at a constant level above his thresholds During threshold measurements, the subject, wearing headphones, was instructed that he was being tested for the "softest" click that he could hear His task was to raise his index finger and keep it raised as long as he could hear clicks in the ear being tested, to lower his finger when the clicks became inaudible, and, then, to raise it as soon as he again could detect the clicks The clicks presented were 1 msec in duration, cycled once per second Each threshold determination began with the clicks well above the subject's threshold and then click intensity was decreased in 1 dB steps until the subject again raised his finger, this complete cycle was repeated 12 times The subject's threshold was defined as the average of the final 10 descending and 10 ascending settings This procedure is modeled on techniques developed by Bekesy (1947) for automatic audiometry

For the dichotic localization task, the subject was instructed that once each second he would hear a click inside his head somewhere between his ears He was told to listen carefully and to signal "right" or 'left" for every second click depending on whether he had heard it to the right or left of the center of his head The subject indicated "right" or left" by manipulating a silent micro-toggle switch which activated signal lights in sight of the experimenter

A particular determination in a given experimental condition began with clicks presented from an intermediate position, one ear or the other leading by 100 or 200 μsec, randomly selected Click intensity in each ear was adjusted to 40 dB above absolute threshold If the subject started off responding "left", his right ear was given a progressive time

advantage ($\simeq$ 5 microseconds/second) until he responded "right" three consecutive times, then his left ear was given the time advantage until three consecutive "left" judgments were obtained This sequence was cycled back and forth 12 times and the mean of the last ten sequences (ten "left" and ten "right" values) was designated the "auditory midline"

This procedure was validated by comparing for 10 subjects auditory midlines obtained with this method with auditory midlines determined using the method of constant stimuli The midlines obtained with the two techniques were not significantly different

*Ambient sound localization*

For ambient localization, the subject was seated in the tilt chair with his head stabilized by the head holder, and the center of the arc carrying the sound source was placed 12 inches above his occipital pole The sound source was set at the zero position (i e, the subject's median plane) and click detection thresholds were determined with the subject listening with both ears, using the bracketing procedure described above During subsequent auditory localiza-[illegible] click intensity was raised 40 dB above [illegible]shold

For the ambient localization task, the subject was told that he would hear clicks coming from somewhere in the coronal plane of his head, once each second, and that he was to indicate by manipulating a toggle switch which way the sound source had to be moved to place it in the median plane of his head The subject was allowed all the time he needed in setting the sound source

Five separate localizations were obtained, each beginning from a different starting position After a localization, the clicks were turned off while the sound source was moved to its next starting position The five starting positions were employed in a counter-balanced order and included left (L) and right (R) placements (relative to the subject's median plane) of 20°L, 10°L, 0°, 10°R and 20°R The mean of the five localizations was designated the "auditory midline".

## Experiment 1

*Influence of body tilt on auditory localization*

Measurements were made of the auditory localizations and perceived posture of tilted subjects to determine whether postural errors and auditory midlocalizations are correlated Both dichotic and ambient localization conditions were included to see whether the direction of auditory displacement would be opposite for dichotic and ambient sounds, as suggested by the results of Teuber and his co-workers (Teuber & Diamond, 1956, Teuber & Liebert, 1956) This experiment was undertaken only after a control experiment failed to reveal differences in click detection thresholds during body tilt, thereby eliminating the possibility that changes in localization might result from peripheral factors

*Procedure* Two groups, each of 12 subjects, made auditory localizations under each of 6 tilt conditions, 28°L, 28°R, 16°L, 16°R, 6°L and 6°R The tilt conditions were presented in a balanced design and were preceded and followed by auditory localizations with the subject upright One group of subjects wore headphones and localized dichotic clicks, the other group localized ambient clicks Each subject was instructed that following a tilt condition he would be returned toward the vertical and when his body felt perfectly upright (i e perpendicular to the floor) he was to tell the experimenter The subject was permitted one correction

After the instructions were completed the subject was fitted with opaque goggles and the experiment was initiated with testing in the first upright condition In the tilt conditions, the subject was told in which direction he would be tilted, but not how far After being tilted, the subject was kept in position for 3 min before testing began Following return to his perceived upright, the subject was

moved to the next tilt position without being told whether he had been upright Consequently, he never gained direct information about the extent or direction of his error in posture

*Results* Errors of opposite sign occurred in the dichotic and ambient localizations of all subjects during tilt The magnitudes of these displacements for a given tilt position were directly related to the postural adaptation effect occurring in that condition, the larger the adaptation to body tilt, the larger the auditory displacement In addition, the size of the postural adaptation to tilt increased with larger tilt angles

Fig 1 illustrates the relationship between dichotic auditory localization and adaptation to body tilt for the various tilt conditions, the auditory shifts are in the same direction as the body tilt (The 6°L and 6°R conditions are omitted because no significant changes either in auditory localization or perceived posture occurred in these conditions, $p > 0.05$) Fig 2 shows corresponding data for the localizations of the ambient source, here the auditory shifts are in the direction opposite the body tilt The figures indicate the

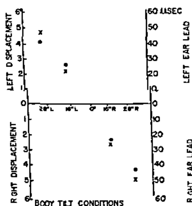



*Fig 1* Relationship between postural adaptation and displacements in dichotic auditory localization Filled circles (*right ordinate*) indicate the necessary lead times to one ear to recenter the sound image when subjects are tilted X s (*left ordinate*) indicate the subjects true orientation in relation to gravity when they perceive themselves as upright following completion of the experimental trial

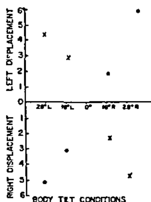



*Fig 2* Relationship between postural adaptation and displacements in ambient sound localization Filled circles (*left ordinate*) indicate the true locus of the sound source in relation to the body during the experimental trial when the sound is perceived to be in the subjective median plane "X's (*left ordinate*) indicate their true orientation in relation to gravity when subjects perceive themselves to be upright upon completion of the experimental trial

auditory cues for which the stimuli are localized in the midline of the body

The effect of starting position on the localizations of subjects performing dichotic localizations was slight, in all cases less than 10 μsec By contrast, the effects of starting position on the localizations of ambient sounds were much larger 3 6°L and 3 9°R for starting positions of 20°L and 20°R, respectively, and 2 1°L and 2 4°R for 10°L and 10°R

*Comment Experiment 1* The experimental observations confirm the reports of Teuber & Liebert (1956) and Teuber & Diamond (1956) that the errors in auditory localization made by blindfolded, tilted subjects are opposite in direction for ambient sounds and dichotically presented sounds These findings also support the notion that auditory direction is dependent upon the registered orientation of the head in addition to the auditory cues at the ears

## *Experiment 2*

### *Time course of auditory displacement during body tilt*

That the magnitudes of the subject's auditory displacements and postural adaptations during

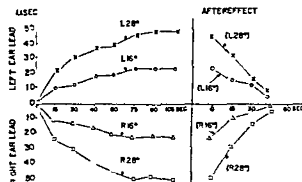



*Fig 3* Changes in the localizations of dichotically presented sounds as a function of elapsed time during and after tilt of the body axis

tilt covary may be a coincidence unless they can be demonstrated to have similar time courses Accordingly, the present experiment was designed to monitor over time these changes

*Procedure* The two groups of subjects from Expt 1 also participated in this experiment Four tilt conditions preceded and followed by testing at the upright, were employed (in a balanced order) 28°R, 16°R, 16°L, and 28°L Measurements of auditory localization were ‑d as soon as the blindfolded subject was ‑ted into position and were continued until the localization values had stabilized The subject was then rapidly returned to the upright (~ 5° sec) and auditory testing was continued

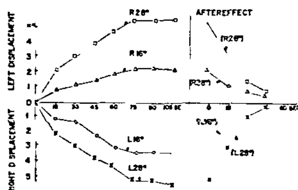



*Fig 4* Changes in the localizations of ambient sounds as a function of elapsed time during and after tilt of the body axis.

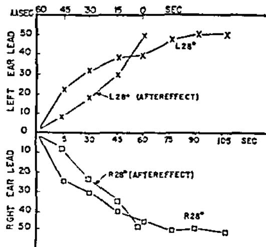



*Fig 5* Comparison of the development of changes in dichotic auditory localization during body tilt with the dissipation of these changes upon return of the body to the gravitational vertical The aftereffects are plotted in relation to the upper time base

until his localizations had again stabilized This procedure was repeated for each tilt condition until all four conditions had been explored

*Results* The results for the dichotic and ambient localization conditions are presented individually in Figs 3 and 4 Auditory displacements developed gradually over time reaching a maximum within 1 ¹/₄ to 2 min After 2 min, no further changes in auditory localization occurred When a subject was returned to the upright, his auditory localizations rapidly returned to normal

Fig 5 illustrates the development of the displacements in auditory localization during body tilt and their decay when the subject was returned to the upright, the decay is much more rapid than the initial development

*Comment Experiment 2* The pattern of these results if dependent on abnormal changes in registered posture requires that a subject when initially tilted rapidly comes to feel less tilted than he actually is and upon being returned to the upright experiences himself as being tilted somewhat to the opposite side

### *Experiment 3*

#### *Time course of postural adaptation during body tilt*

In conjunction with the results of Expt 2 this experiment should establish whether abnormal changes in registered posture are the determining factors in the auditory displacements that occur during tilt

*Procedure* The same subjects, tilt conditions (28°L, 28°R, 16°L, 16°R) and order of conditions were used as in Expt 2, although no auditory localizations were involved

Each subject was instructed that he would be tilted and held in position for varying periods after which he would be returned toward the vertical, he was to concentrate carefully and report when he again experienced himself to be upright The experimenter then tilted the blindfolded subject into position for the appropriate time (15 sec, 30 sec, 45 sec, 60 sec, 75 sec, 90 sec, or 105 sec), returned him toward the upright noted the value at which he reported himself upright, and returned him to the true upright When the subject again reported himself upright, he signalled the experimenter who recorded the time elapsed from the subject s actual return to the upright This procedure was repeated for each tilt condition

*Results* The experimental results for both groups are presented in Fig 6 When compared with the findings of Expt 2, it is clear that the time course of the auditory displacements during body tilt and the time course of the postural adaptations are virtually identical The length of time a subject is tilted before his auditory localizations stabilize is dependent on the time it takes for his postural adaptation to reach a maximum The size of the final (end point) auditory localization displacements obtained in a given tilt condition in Expt 2 is not significantly different from the size of the adaptation effect to tilt observed in the comparable condition in Expt 3 ($p > 0\ 05$)

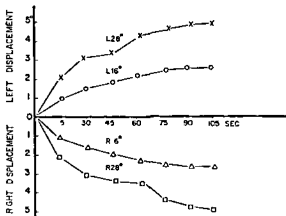



*Fig 6* Development of postural adaptation as a function of body tilt and elapsed time The ordinate specifies the angle of the body in relation to the gravitational vertical upon return to the perceived upright

As they were returned toward the vertical, all subjects reported themselves to be upright when they were still tilted to the same side, upon reaching the true upright, they reported themselves to be tilted somewhat to the opposite side The time elapsed after the subjects were returned to the upright before they reported feeling upright was not significantly different from the duration of the aftereffects in auditory localization found in Expt 2 ($p > 0\ 05$)

*Comment Experiment 3* Taken together the results of Experiments 1, 2, and 3 implicate postural adaptation effects as being responsible for the auditory displacements that occur during body tilt Thus, a subject s auditory localizations are made with respect to his orientation as registered by his central nervous system even though his body may in fact be more tilted

### *Experiment 4*

#### *Auditory localizations during body tilt of subjects with unrestricted vision*

Ordinarily, vision is our most important sense, since most orientation and movement take place under visual guidance Therefore, it is of interest to determine whether subjects per

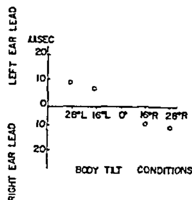



*Fig 7* Changes in dichotic auditory localization as a function of body tilt in subjects with unrestricted vision The ordinate indicates the necessary relative time advantage to one ear to produce a centered sound image

mitted visual information concerning their true body orientation will show postural adaptation effects and auditory displacements during body tilt

*Procedure* The experimental design was identical to that of Expt 1, except that subjects were permitted unrestricted vision, consequently, at all times they had information from an articulated visual environment about their true orientation relative to the vertical

Only dichotic localization was used in this [illegible]riment, so that there would be no pos[illegible]lity of the subject seeing the sound source

*Results* Fig 7 presents the experimental findings Significant changes in auditory localization did not occur ($p > 0.05$)

*Comment Experiment 4* The results suggest that the reference frame for orientation is set primarily by the visual input and that other modalities serve to establish the reference coordinates primarily in the absence of vision

## *Experiment 5*

### *Influence of prior visual information on auditory localization during body tilt*

Although the presence of visual information leads to accurate auditory localizations during tilt, it is important to determine when vision is restricted *after* the subject is in position, whether the subject will revert to the use of proprioceptive-vestibular tactile information about his body orientation or whether he will be able to "remember" his position *(as specified by the prior visual information)* and maintain veridical performance

*Procedure* The design was similar to that of Expt 4 except that within each tilt condition a subject was blindfolded and immediately retested after his auditory localizations had been measured the first time Then, the blindfold was removed and the subject with his vision unrestricted was returned to the upright, whereupon he closed his eyes and his dichotic auditory localizations were remeasured

*Results* From Fig 8 it can be seen that as long as a subject's eyes were open, his auditory localizations remained stable and veridical, however, as soon as he closed his eyes, his auditory localizations assumed values closer to those found in experiments in which the subjects were blindfolded throughout In the aftereffect condition, there were small, non significant displacements in auditory localization

*Comment Experiment 5* This experiment demonstrates that as long as visual informa

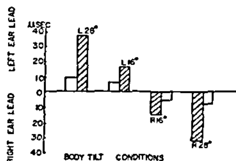



*Fig 8* Changes in dichotic auditory localization after 3 min of body tilt with eyes open Empty bars indicate localizations with eyes open hatched bars indicate localizations with eyes closed Subjects are tilted during the measurements

tion is available concerning the true extent of body tilt it "dominates" other sensory inputs in aligning the spatial "reference frame" used in localization When vision is excluded the remembered knowledge of true body orientation is not sufficient to maintain veridical localization and other sensory sources are progressively relied upon

*Experiment 6*

*Postural adaptation effects in tilted subjects with unrestricted vision*

The pattern of results just described raised the question whether visual knowledge of "true" body orientation and nonveridical postural information from other modalities could be maintained simultaneously

*Procedure* The design of Expt 6 is similar to that of Expt 3, except that a subject was tilted with his eyes open He remained in his tilted position for three minutes, then was blindfolded and returned toward the upright The experimenter noted the point at which the subject reported being upright, returned him to the true upright, and recorded the elapsed time before the subject again reported himself to be upright.

*Results* Subjects showed significant postural adaptation effects ($p<0.05$) but of a much smaller size than those observed in experiments in which the subjects were blindfolded throughout the experimental procedure The results are illustrated in Fig 9

*Comment Experiment 6* Even when a tilted subject has visual information concerning his body orientation small, postural adaptation effects are developing in the representation of his body orientation as specified by other sense modalities If the visual information is removed the subject relies on vestibular or proprioceptive "misinformation" to assess his body orientation It is clear, however, that the magnitude of the postural adaptation effects developing in these other modalities is attenuated in the presence of visual input

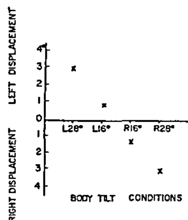



*Fig 9* Magnitude of postural adaptation after 3 min of body tilt with eyes open The ordinate indicates true body orientation when subjects perceive themselves as being upright upon being returned (with eyes closed) toward the gravitational vertical

## DISCUSSION

The ability to localize a sound in space requires a reference system by which the auditory cues at the ears are assigned to various external directions In conjunction with information about the position of the head in relation to the trunk (and the head relative to gravity) this spatial reference system permits judgments about the direction of a sound source with respect to the head or torso The experiments that have been described systematically investigated the interrelation between this reference system and positional information about body orientation

Specific predictions were made concerning the interaction of postural and auditory cues in the establishment of auditory direction In particular, it was predicted that changes in perceived posture should be reflected by changes in auditory direction of similar magnitude and time course, and, moreover, that in keeping with the findings of Teuber & Liebert (1956) and Teuber & Diamond (1956), the direction of auditory change should be opposite

for dichotic and ambient signals All of these predictions were supported by the outcome of the experiments

Although the observations of Teuber & Diamond (1956) and Teuber & Liebert (1956) that during body tilt the direction of displacement for ambient and dichotic sounds is opposite were replicated, the reason for this difference is unclear An analogous situation seems to hold, however, for vision

Roman et al (1963) and Warren et al (1963) have reported that the apparent displacements of afterimages and external visual targets during the 0 g (weightless) phase of parabolic flight are in opposite directions During 0-g, as in the present paradigm involving body tilt, an abnormal change in perceived posture occurs and it is possible that the opposite signs of the displacements are related to whether the stimulus is interpreted as being 'anchored' to the subject and thus "moving" with the subject's changed interpretation of his position (e g , dichotic clicks presented via headphones afterimages) or as being independent of his motion (e g , ambient clicks, external visual targets) Additional experimentation is clearly necessary to refine this distinction, but can nevertheless be concluded on the basis of the present experiments that not only the auditory cues at his ears but also information about his orientation in space determine where a subject will hear a sound

## ACKNOWLEDGMENT

I thank Professor H L Teuber for his valuable comments and encouragement

## ZUSAMMENFASSUNG

Der Unterschied zwischen der wahren und der von einer Versuchsperson selbst wahrgenommenen Orientierung ergibt beim Horen Fehllokalisierungen ahnlichen Grossen und Zeitausmasses Gewisse Stutzpunkte der visuellen Umwelt jedoch verhindern die Entwicklung von falschen Interpretationen der eigenen Körpherhaltung und dardurch auch der auditorischen Lokalisierung Diese Beobachtungen werden als Beweis fur ein raumliches Referenzsystem angesehen das fur die Erhaltung einer Richtungskonstanz beim Horen und Sehen verantwortlich ist Sie zeigen weiter dass die Position des Horens nicht nur von den ans Ohr dringenden Lauten abhangt sondern auch von der selbst wahrgenommenen eigenen Korperorientierung

## REFERENCES

Békesy, G von 1947 A new audiometer *Acta Otolaryng* (Stockh ) *35* 411

Clark B & Graybiel A 1963 Perception of the postural vertical in normals and subjects with labyrinthine defects *J Exp Psychol 65* 490

Fleishman E 1953 Perception of body position in the absence of visual cues *J Exp Psychol 46* 261

Hay, J Pick H & Ikeda K 1965 Visual capture produced by prism spectacles *Psychon Sci 2* 215

Lackner J 1970 Prism adaptation affects auditory midline Paper read to East Psychol Assoc Atlantic City

— 1973 Visual rearrangement affects auditory localization *Neuropsychol 11* 29

Mann C & Passey G 1951 The perception of the vertical V Adjustment to the postural vertical as a function of the magnitude of postural tilt and duration of exposure *J Exp Psychol 41* 108

Mann C & Ray J 1956 The perception of the vertical XIV The effect of rate of movement on the judgment of the vertical Proj NM 001 110 500 Rep No 40 *U S Nav Sch Aviat Med Res Rep* Pensacola Fla

Nielsen T 1963 Volition a new experimental approach *Scand J Psychol 4* 225

Passey G & Guedry F 1949 The perception of the vertical II Adaptation effects in four planes. *J Exp Psychol 39* 700

Roman J Warren B Niven J & Graybiel A 1963 Some observations on the behavior of a visual target and a visual afterimage during parabolic flight maneuvers *Aerosp Med 34* 841

Teuber H L & Diamond S 1956 Effects of brain injury on binaural localization of sounds Paper read at East Psychol Assoc , Atlantic City

Teuber H L & Liebert R 1956 Effects of body tilts on auditory localization *Amer Psychol 11* 430A

Teuber H L & Mishkin M 1954 Judgment of visual and postural vertical after brain injury *J Psychol 38* 161

Warren B Roman J & Graybiel A 1963 Exclusions of angular accelerations as the principal cause of visual illusions during parabolic flight maneuvers Joint Report No SAM TDR-63 78 *USAF Sch Aviat Med Res Rep* Brooks AF Base Tex and *US Nav Sch Aviat Med Res Rep* Pensacola Fla Proj MR005 13-6001 Rep No 85

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# EFFECT OF CONTRALATERAL BROAD BAND NOISE ON FREQUENCY DISCRIMINATION

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*Abstract* The effect of contralateral masking on a person s ability to detect small changes in frequency was studied Eight normal hearing young adults with no previous participation in psychoacoustic experiments served as subjects The task consisted of matching the frequency of a variable signal (3 575–4 425 Hz) to that of the reference tone (4 000 Hz) by the method of adjustment As many tone pairs as necessary to complete the judgment were presented in a fixed temporal pattern All subjects performed the task twenty-four times at both 20 and 40 dB SL with and without 40 dB SL of contralateral noise for a total of 96 judgments The results indicated that contralateral broad band noise significantly improved the subjects proficiency as a group to detect small changes in frequency although intersubject differences were noted The presentation level of the reference and variable signals was shown to have little or no effect on scores

The effects of contralateral masking on two site of lesion tests—Bekesy audiometry and the SISI—have been explored by Blegvad & Terkildsen (1966, 1967) Blegvad (1968 1969) Tice (1968) and Osterhammel et al (1970) The results of those studies indicate that this form of masking can influence significantly the listener s differential sensitivity for changes in intensity

Interest in monaural and binaural frequency discrimination as a promising diagnostic tool has been rekindled recently by Campbell (1970) Jones & Pracy (1971) and Carhart (1973) The Frequency Increment Sensitivity Test (FIST) developed by Campbell (1970) has been suggested as another method which may be used to differentiate between normal and pathological ears Based on a limited number of subjects his data indicate that FIST is able to "differentiate between normal and damaged cochleae (p 376) In reviewing his results Campbell reports that further investigations need to be conducted on several aspects of his procedure before it can be utilized routinely as a clinical tool A recommendation of particular interest is that of studying the effect of contralateral masking on FIST scores This concern is not limited to the FIST procedure alone but is a valid question with any measure of frequency discrimination

The purpose of this study was to determine whether contralateral masking would influence the proficiency of normal hearing subjects in detecting small changes in frequency Secondarily, we wished to explore sensation level effects on frequency discrimination at 4 000 Hz with and without the presence of contralateral masking

## MATERIAL

### *Subjects*

Eight young adults between the ages of 20 and 30 years ($\overline{X} = 27.2$) were selected based on the following criteria (1) normal hearing

Table II *Group means and standard deviations in Hertz of the difference between the reference and variable stimuli*

| Observation | Without contralateral noise Presentation level | | With contralateral noise Presentation level | |
|---|---|---|---|---|
| | 20 dB SL | 40 dB SL | 20 dB SL | 40 dB SL |
| Mean | 23 6 | 22 4 | 20 7 | 20 3 |
| Standard deviation | 21 1 | 19 2 | 18 8 | 19 6 |

rise and decay time of 50 msec was employed for both the reference and variable signals An inter stimulus interval of 500 msec separated the reference signal from the variable signal Successive pairs of tones were presented every 1 500 msec

*Test presentation order*

All subjects were interviewed and received hearing evaluations prior to the experimental test session Frequency discrimination measurements were obtained for 4 000 Hz in the right ear under the following conditions (1) at 20 and 40 dB SL without contralateral noise, and (2) at 20 and 40 dB SL with 40 dB SL of contralateral noise Each subject made a total of ninety six judgments that is, twenty-four judgments for each of the four stimulus conditions The sequence of each condition was varied systematically to preclude order and practice effects from biasing test results

## RESULTS

The data were analysed by means of analysis of variance employing a three factor approach for repeated measures (Winer, 1962) As indicated by the data in Table I, there was a significant difference between subjects in their ability to match the frequency of successive pairs of tones at 4 000 Hz ($p=0\ 01$) Furthermore, frequency matching at 4 000 Hz was not significantly affected by varying the sensation level at which the stimuli were presented However, it was shown that the presence or absence of contralateral noise can significantly affect the size of the difference score ($p=0\ 05$) By looking at the mean scores and standard deviations for all subjects as a group in Table II, it can be seen that frequency matching differences were smaller for the conditions in which contralateral noise was applied to the non test ear Also it should be noted that even though contralateral masking did improve the subject's ability to detect small changes in frequency as a group, this was not evidenced for each individual subject (Table III) Finally, there was no significant interaction exhibited between the variables

As a point of interest, a corollary statistical analysis was performed on the number of paired stimuli required for the subjects to make a judgment under each of the conditions Table IV indicates that neither presentation level nor contralateral masking significantly influenced the number of stimuli required to make a judgment ($p=0\ 05$) However, there was a significant difference between subjects regarding the actual number of stimuli required to make a judgment ($p=0\ 01$) The means and standard deviations for the number of stimuli presented under each condition are included in Table V

In summary, it can be said that contralateral broad band noise did improve the subjects proficiency, as a group, to detect small changes in frequency ($p=0\ 05$), while presentation level was shown to have little or no effect on their scores within the context of this design Also it is clear that there were significant differences between subjects in their ability to match

Table III *Individual means and standard deviations in Hertz of the difference between the reference and variable stimuli*

| Subject | Observation | Without contralateral noise Presentation level 20 dB SL | 40 dB SL | With contralateral noise Presentation level 20 dB SL | 40 dB S |
|---|---|---|---|---|---|
| 1 | Mean | 23.7 | 23.6 | 27.7 | 22.3 |
| | SD | 15.0 | 20.9 | 21.0 | 16.7 |
| 2 | Mean | 13.0 | 11.1 | 12.5 | 10.0 |
| | SD | 7.4 | 7.2 | 9.4 | 8.8 |
| 3 | Mean | 45.3 | 34.3 | 34.9 | 41.0 |
| | SD | 33.7 | 20.8 | 25.3 | 28.0 |
| 4 | Mean | 9.7 | 15.2 | 11.7 | 15.4 |
| | SD | 8.5 | 13.6 | 8.8 | 13.7 |
| 5 | Mean | 20.8 | 23.1 | 19.3 | 17.0 |
| | SD | 14.6 | 16.5 | 16.1 | 16.4 |
| 6 | Mean | 25.0 | 21.2 | 13.8 | 17.8 |
| | SD | 16.4 | 13.6 | 12.5 | 14.6 |
| 7 | Mean | 31.9 | 36.1 | 29.2 | 21.0 |
| | SD | 23.2 | 24.9 | 20.8 | 17.0 |
| 8 | Mean | 19.1 | 14.8 | 16.1 | 17.7 |
| | SD | 13.8 | 13.8 | 13.8 | 19.7 |

the frequency of paired stimuli at 4 000 Hz ($p = 0.01$)

## DISCUSSION AND CONCLUSIONS

### *Comparison with results of previous studies on DLFs in normal-hearing subjects*

The difference limina for 4 000 Hz obtained by Harris (1952), Konig (1957), and Parker et al (1968) can be compared to the results obtained in this investigation by studying the data presented in Table VI Even though the subject's task and the psychophysical methods differ, the mean or median scores are quite similar There is considerable divergency in data, however, between the standard deviations for the designs utilizing the method of adjustment and Konig's (1957) study which employed the method of constant stimuli Whether this discrepancy is attributable to the differences in design or subjects, cannot be resolved within the limits of this study

It is also interesting to note that Konig (1957) reports smaller DLF's as sensation level is increased from 10 to 40 dB He failed to mention whether these differences were statistically significant Parker et al (1968) did not find statistically significant differences between scores obtained at 10 and 40 dB SL, nor did the present study find significant differences between scores obtained at 20 and 40 dB SL Brandt (1963), in a detailed study of the DLF at 1 000 Hz, found statistically significant improvements in frequency difference limina only as sensation level was increased from 0 to 5 dB Above 5 dB SL, improvements in DLF as a consequence of increases in sensation level, were non-significant. The implications are that increases in sensation level of 10 dB or more do not produce smaller frequency difference limina, at least for the test paradigm used in this study and by Parker et al (1968)

### *Neural interaural interdependence*

Numerous studies in audition have demonstrated the presence of neural interaural interdependence (Egan, 1948, Reynolds & Stevens, 1960, Prather, 1961, Vigran, 1965, Blegvad & Terkildsen, 1966, 1967, Blegvad, 1968, 1969, Tice, 1968, Osterhammel et al,

Table IV *Summary of analysis of variance for all subjects as a group on the number of stimuli required to make a judgment utilizing two presentation levels, with and without the presence of contralateral masking*

A = Subject
B = Presentation level
C = Noise level

| Source of variance | | Degrees of freedom | Sum of squares | Mean square | *F* |
|---|---|---|---|---|---|
| *Between observations* | | 191 | 18 726 801 | | |
| | A | 7 | 13 509 686 | 1 929 955 | 68 066* |
| Error | A | 184 | 5 217 115 | 28 354 | |
| *Within observations* | | 576 | 12 388 750 | | |
| | B | 1 | 57 970 | 57 970 | 3 034 |
| | AB | 7 | 190 207 | 27 172 | 1 422 |
| Error | B | 184 | 3 515 073 | 19 104 | |
| | C | 1 | 9 408 | 9 408 | 0 495 |
| | AC | 7 | 156 603 | 22 372 | 1 178 |
| Error | C | 184 | 3 494 239 | 18 990 | |
| | BC | 1 | 4 230 | 4 230 | 0 166 |
| | ABC | 7 | 275 155 | 39 308 | 1 543 |
| Error | BC | 184 | 4 685 865 | 25 467 | |

* = significant at 0 01

1970 Rowley & Studebaker, 1971) Prather (1961) and Rowley & Studebaker (1971) support the contention that an increase in loudness with presence of contralateral noise is the result of central summation These authors do not offer concrete evidence, however that would easily allow the researcher to accept their supposition as unequivocal Vigran (1965) similarly reports that an increase in loudness can be associated with the presence of contralateral noise He found that there was a greater increase in the loudness of a tone in the test ear when narrow bands of noise centered around the frequency of the test tone were used to mask the non test ear

On the contrary Tice (1968) investigated the effects of contralateral narrow-band noise on the SISI He hypothesized that contralateral narrow-band noise, as compared to broad band noise, would increase the proficiency of listeners to detect small changes in intensity He reasoned that an increase in the facilitation effect with contralateral narrow band noise would strengthen the notion that this increased proficiency was the sequela of binaural summation Indeed, Tice did find an increase in the proficiency of listeners to detect small changes in intensity with the introduction of contralateral noise There was not however, a significant difference between SISI scores obtained with broad band and narrow-band contralateral noise Consequently, Tice was not able to draw conclusions similar to those reported by Vigran (1965) Paul & Barry

Table V *Group means and standard deviations for the number of paired stimuli that needed to be heard in order to make a judgment*

| | Without contralateral noise Presentation level | | With contralateral noise Presentation level | |
|---|---|---|---|---|
| Observation | 20 dB SL | 40 dB SL | 20 dB SL | 40 dB SL |
| Mean | 10 5 | 10 1 | 10 4 | 9 7 |
| S D | 7 0 | 6 3 | 6 5 | 5 6 |

Table VI *A comparison of pitch matching scores and DLFs for 4 000 Hz in normal hearing subjects*

| Study | Mean | S D | Presentation level | No of subjects | Psychophysical method | Subject s task |
|---|---|---|---|---|---|---|
| Labiak (1971) | 22 4[a] | 19.2 | 40 dB SL | 8 | Adjustment | Equal |
| Harris (1952) | 21 0[b] | Not given | 30 dB SL | 52 | Constant stimuli | Higher or lower |
| Konig (1957) | 21 2 | 6 8 | 40 dB SL | 10 | Constant stimuli | Higher or lower |
| Parker et al (1968) | 18 5 | 19.3 | 40 dB SL | 50 | Adjustment | Equal |

[a] 40 dB SL without contralateral noise data only
[b] Median score

(1972) state that with the introduction of contralateral masking, detection of increments can be adversely affected Based on their data, Paul & Barry speculate that the first few increments in a series may be less detectable due to an initial increase in neural activity level, but additional increments will be enhanced by a subsequent reduction in this activity It would appear, then, that even though a facilitation effect has been shown to exist in the detection of small changes in frequency and intensity, the basis of the phenomenon cannot be defined clearly at the present time

*Clinical and research implications*

Various studies (Meurman 1954, Butler & Albrite, 1956, Parker et al, 1968) have compared the frequency discrimination capacity of subjects with normal and pathologic ears None of these studies employed contralateral masking to eliminate the possibility of cross conduction of the test stimulus In fact, only one previous study (Campbell, 1970) has mentioned contralateral masking in relation to frequency discrimination scores Thus it seems logical to conclude that any future clinical research conducted on frequency discrimination should consider the possible influence of contralateral masking In addition, since the design of this study was limited to one level of contralateral noise, one frequency, and a limited population, future investigations should explore these parameters in greater detail

It is also recommended that caution be exercised regarding the choice of psychoacoustic methodology to be employed while evaluating frequency discrimination Harris (1952), Konig (1957), and Parker et al (1968) contend that the frequency modulation technique, often used in clinical situations, is not a valid measure of frequency discrimination due to the inherent artifacts introduced with this procedure It is suggested that the method of constant stimulus or method of adjustment in combination with successively paired tone bursts be adapted for clinical investigations

Although there is still a paucity of data regarding the effects of various pathologies on frequency discrimination, our knowledge is increasing This study and previous investigations have suggested possible test paradigms and associated precautions for evaluating these phenomena Hopefully, an in depth resurgence in the study of this aspect of audition will prove to be clinically rewarding

## ACKNOWLEDGMENT

The authors express their appreciation to Dr Wendel Walton for his assistance in instrumenting this study

Table IV *Summary of analysis of variance for all subjects as a group on the number of stimuli required to make a judgment utilizing two presentation levels, with and without the presence of contralateral masking*

A = Subject
B – Presentation level
C = Noise level

| Source of variance | | Degrees of freedom | Sum of squares | Mean square | *F* |
|---|---|---|---|---|---|
| *Between observations* | | 191 | 18 726 801 | | |
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| | B | 1 | 57 970 | 57 970 | 3 034 |
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| | C | 1 | 9 408 | 9 408 | 0 495 |
| | AC | 7 | 156 603 | 22 372 | 1 178 |
| Error | C | 184 | 3 494 239 | 18 990 | |
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| Mean | 10 5 | 10 1 | 10 4 | 9 7 |
| S D | 7 0 | 6 3 | 6 5 | 5 6 |

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# SUBMIKROSKOPISCHE VERANDERUNGEN DER REISSNERSCHEN MEMBRAN NACH STREPTOMYCINAPPLIKATION

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(Eingegangen am 26 April 1973)

*Abstrakt* Wir beobachteten in den Epithelial , aber auch Mesenthelialzellen eine große Aktivitat und neben der Beeintrachtigung der normalen Aktivitat die Zerstorung der Zellen von mesenthelialer Seite her Es laßt sich sagen, daß die RM entsprechend Versuchsdauer und angewandtem Antibiotikum sowie Dosierung nach einem Gradienten von der Peri- zur Endolymphe hin zerstort wird da die Pinozytose von der Basalmembran ihren Ausgang nimmt und zu einer deutlichen Vakuolisierung der Mesenthelialzellen fuhrt Desgleichen sind die Veranderungen im Bereich der Zonula occludens und des Desmosomen von Bedeutung

Die Reissnersche Membran (RM) ist fur das Innenohr von besonderer Bedeutung, da sie als biologische Membran sowohl aktiv als auch passiv die Austauschvorgange zwischen Peri- und Endolymphe beeinflußt

Submikroskopische Untersuchungen der normalen Struktur der RM wurden wiederholt durchgefuhrt (Robertson, 1959, Hagiwara, 1963, Iurato, 1967, Duvall & Sutherland, 1970, Iurato & Taidelli, 1971) Sie besteht aus zwei unterschiedlichen Zellagen, die durch eine Basalmembran voneinander getrennt sind Die Bindegewebszellen auf der Seite der Scala vestibuli sind zart, Mikrovilli fehlen Die der Scala media zugewandte epitheliale Zellage ist durch sogenannte Mikrozytosevesikel, einen gut entwickelten Golgiapparat im Zytoplasma und zahlreiche Mikrovilli zur Endolymphe hin gekennzeichnet Watanuki beobachtete, daß die Epithelialzellen zum Limbus spiralis hin schmaler sind als die zum Ligamentum spirale

Die Pathologie und Histochemie der Schadigung des Innenohres durch Streptomycin praparate wurden, wie die Untersuchungen von Peri- und Endolymphe, wiederholt beschrieben (Kohonen, 1965, Musebeck & Schatzle, 1962, etc ) Wie man glaubt, sind die Wirkungen des Streptomycins auf die lebende Zelle folgende

1 Beeintrachtigung der Funktion der Zellmembran und

2 Hemmung der Proteinsynthese der Zelle

Die primare Schadigung von Dihydrostreptomycin (DHS) lokalisiert sich in den Mitochondrien (Duvall & Wersall, 1964, Musebeck, 1963) oder in den Ribosomen (Spoendlin, 1966) und entspricht im wesentlichen den von Brock (1966) gefundenen Einwirkungen von Antibiotika auf Bakterien

Die angefuhrten Änderungen der normalen Strukturen erscheinen als Resultat der Einfuhrung der Antibiotika in die Endolymphe uber die Stria vascularis und die RM

Submikroskopischen Untersuchungen der Reissnerschen Membran nach Streptomycinapplikation kommt eine besondere Bedeutung zu In der Literatur fehlen diese Untersuchungen (Spoendlin et al , 1971)

Herrn Prof Dr sc med Kurt Dietzel zum 60 Geburtstag gewidmet.

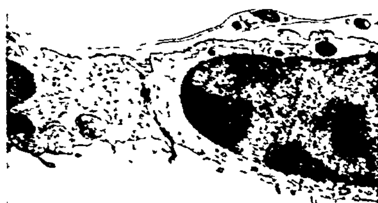


*Abb 1* RM nach der im. Injektion von 10×250 mg/kg Korpergewicht Streptomycinsulfat. In den epithelialen Zellen finden sich fingerartige Fortsatze, die sich zur Seite der verdickten Basalmembran hin erstrecken Beginnende Vergroßerung des Zwischenzellspaltes im Bereich des Desmosomen, der Zona occludens. Beginnende Vakuolisierung und Vergroßerung der Lysosomen der mesenchymalen Zellen 9 100 1

## MATERIAL UND METHODE

Zwanzig ohrgesunde Meerschweinchen, etwa 250 g schwer, erhielten nach der Prufung mittels Preyerschem Ohrmuschelreflex 250 mg Streptomycinsulfat/kg Korpergewicht/die Nach 5, 10, 15 und 20 Tagen wurden je 5 Tiere der Versuchsserie entnommen und beide Cochlea nach der Methode von Vinnikov & Titova (1964) gewonnen Alle Cochleae wurden nach sofortiger Fixierung in 1%iger Os- und der Aufarbeitung nach der Methode von Kolfield in Epon eingebettet, die Ultradunnschnitte mit dem Ultrotom III (LKB Stockholm) angefertigt und mit dem Elektronenmikroskop JEM 6 c untersucht

## UNTERSUCHUNGSERGEBNISSE

Die ersten auffälligen Veränderungen an der RM wurden nach 10 Injektionen beobachtet (Abb 1) In den epithelialen Zellen kommen fingerartige Fortsatze zur Beobachtung, welche sich zur Seite der Basalmembran hin erstrecken Außerdem sind Verdickungen der Basalmembran zu beobachten Auf der Seite der Basalmembran sieht man eine verstarkte Neubildung von Lysosomen An den Kontaktstellan zweier Zellen in dem Gebiet des Desmosomen, der Zonula occludens kommen deutliche Vergrößerungen des Zwischenzellspaltes zur Beobachtung Die der Scala vestibuli zugewandte Seite der Zelle befindet sich jedoch in einem relativen Zustand der Ruhe In den mesenchymalen Zellen beobachtet man eine Vergrößerung der Lysosomen und eine beginnende Vakuolisierung Zwischen benachbarten Zellen sieht man größere Lücken mit Flussigkeit. Im Bereich dieser findet sich eine Hyperplasie des Desmosomen An der vestibulären Seite der Epithelialzelle nimmt anscheinend vorubergehend die Zahl der Mikrovilli zu Im Zytoplasma werden große Lipidgranula beobachtet (Abb 2) Sehr demonstrativ sind die Veränderungen nach dem 15 Tag In diesem Stadium beobachtet man eine Zunahme der Flussigkeit zwischen den Epithelialzellen Im Bereich zwischen Epithelial- und Mesenchymalzelle kommen vereinzelt Ausfälle von Niederschlagen zur Beobachtung An der Stelle, an welcher die Epithelialzellen miteinander Kontakt haben, vergroßert sich der Durchmesser des Zwischenzellspaltes, aber die Desmosomen und die Zona occludens bleiben Sehr deutlich sind die Erscheinungen von Vakuolisierung und Pinozytose, Mikrozytosevesikel vermindern sich (20 Versuchstag, Abb 3)

In den Mesenchymalzellen nehmen Zahl und Große der Lysosomen zu Außerdem sieht man auch in diesen Zellen eine deutliche Vakuolenbildung

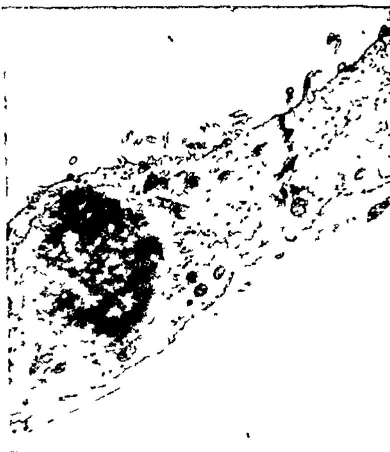


*Abb 2* RM nach der im Injektion von 15 × 250 mg/kg Korpergewicht Streptomycinsulfat Es zeigen sich starke Lipdanhaufungen im Zytoplasma der Epithelialzelle und eine Hyperplasie des Desmosomen 13 800 1

Nach dieser Versuchsdauer sind Verschmelzungen der Basilarmembran, Polysomenanhaufungen (Abb 4), Vermehrung von freien Ribosomen (Abb 5) sowie eine weitere Abnahme von Mikrozytosevesikeln nachweisbar und eine Vergroßerung der Lysosomen besonders auf der den mesenchymalen Zellen zugewandten Seite (Abb 6) Die Zellorganellen werden zunehmend elektronenoptisch dunkler

Alle diese beobachteten Faktoren deuten darauf hin, daß es unter den gegebenen Versuchsbedingungen zu einer starken Veranderung der Permeabilitat von der Peri zur Endolymphe hin kommt Wir glauben, daß es nach der Gabe von Antibiotika primar zu Permeabilitatsstorungen durch Veranderungen in den Zellmembranen kommt, wahrend die pathologischen Veranderungen in der Zellen sekundarer Natur sind

## DISKUSSION

Das Streptomycin beeintrachtigt die Permeabilitat der RM stark Die pathologischen Veranderungen, die diese Beeintrachtigung hervorrufen haben einen progredienten Faktor, der sich offensichtlich von der Peri zur Endolymphe hin ausbreitet Diese Schlußfolgerungen ergeben sich aus den vorliegenden Ergebnissen

Beobachtet werden eine ausgepragte Pinozytose, eine teilweise Verschmelzung der Basalmembran zwischen Epithelial und Mesothelialzellen Luckenbildung im Bereich der Basalmembran Die Zwischenzellspalten vergroßern ihren Durchmesser, fullen sich mit Flussigkeit an, wahrend die Desmosomen und die Zonula occludens erhalten bleiben Auf Grund unserer Untersuchungen mochten wir entsprechend Duvall et al (1969) and Duvall



*Abb 3* RM nach der im Injektion von 20×250 mg/kg Korpergewicht Streptomycinsulfat Zwischen den Mesenthelial und Epithelialzellen werden Aus flockungen beobachtet weiter starke Lipidanhaufun gen sowie eine Vakuolisierung und Pinozytose im Zy toplasma der Epithelialzellen 21 000 1

& Sutherland (1970) zwei potentielle Moglichkeiten des Durchdringens der RM von der Peri- zur Endolymphe annehmen 1 durch die Pinozytose und 2 über die Zwischenzell spalten Eine zunehmende Vakuolisierung sowie eine Verminderung von Mikrozytoseblaschen und zunehmend elektronenoptisch dunkler werdenden Zellorganellen erinnern an die von Kaneko et al (1970) und Hawkins (1970) erhobenen Befunde Eine unterschiedlich starke elektronenoptische Dichte des Zytoplasmas, wie sie Kaneko et al (1970) nach der Gabe von Kanamycin, welche zur Differenzierung der RM in dunkle und helle Zellen fuhrte, sahen, konnte von uns, ahnlich wie Hawkins (1972), nicht beobachtet werden Ciges et al (1972) sahen ein verandertes Aufnahmevermogen von Neutralrot der RM nach Kana mycinintoxikation

Hervorgerufen werden diese pathologischen Prozesse durch eine Zunahme der Konzentra tion des Streptomycins primar in der Peri lymphe, wodurch es schließlich zu einer Zerstorung der membranosen Strukturen der Zellen kommt

In Zusammenhang mit der fortschreiten den Zerstorung der membranosen Struktur der Zellen, dem Verschwinden der SDH nach Streptomycingabe und dem Schutzmechanis mus des Cytochrom *C* muß man auch eine Beeintrachtigung des Sauerstoffmetabolismus bei Streptomycinintoxikation als gesichert an sehen

Durch die steigende Konzentration des Praparates in der Perilymphe wird die RM ge schadigt, wodurch ihre Funktion als effektive biologische Trennmembran zwischen Peri und Endolymphe beeintrachtigt wird

Die zwischen den Epithelial und Mesenthelialzellen beobachteten Niederschlage konnten durch eiweißartige Flockungen oder — wie Schatzle (1972) meint — durch das Streptomycin ausgefallte saure Mucosaccharide be dingt sein

Nach Kanamycinbehandlung, Hypoxie und Larmbelastung kommt es zu einer Permeabi



*Abb 4* RM ebenfalls nach 20 Injektionen (s o ) Besonders deutliche Verminderung der Mikrozytosevesikel des Zytoplasmas Ausflockungen zwischen beiden Zellagen sowie ein auffallend breiter Zwischenzellspalt benachbarter Epithelialzellen und Polysomenanhaufungen sind nachzuweisen 23 000 1

litatssteigerung der RM (Misrahy et al , 1962) Von Stupp (1971) wird unter ahnlichen Versuchsbedingungen zwar eine deutliche Zunahme der Kaliumkonzentration in der Perilymphe bestatigt als Ursache aber nicht unbedingt eine Verminderung des aktiven Kationentransportes durch die RM diskutiert Moglich ware auch eine scheinbare Hemmung des Kaliumdurchtrittes durch die RM als Folge einer verminderten Resorptionsfahigkeit der Stria vascularis, wodurch zwangslaufig der Flussigkeitsstrom und Ionentransport von der Scala tympani durch die RM zum Endolymphraum hin zum Erliegen kommen konnte

Die histochemischen Untersuchungen des Innenohres auf Proteinbausteine von Musebeck & Schatzle (1962) ergaben nach den Methoden von Danielli und Burstone eine mittelgradige, positive Reaktion der RM Die respiratorische Rate der RM verglichen mit anderen Geweben ist außerordentlich hoch

Der Eiweißstoffwechsel der RM gemessen durch die Bestimmung der Eiweißenbaurate betragt im Gegensatz zur hohen respiratorischen Rabe nur 50% des Eiweißstoffwechsels der Ganglienzellen und liegt auch unter dem der Stria vascularis Watanuki et al (1968 1968) untersuchten den RNS-Stoffwechsel der Zellen der RM und schlußfolgern auf Grund unterschiedlicher Ergebnisse fur die inneren mediolusnahen und die außeren



*Abb 5* RM ebenfalls nach 20 Injektionen (s o ) Freie Ribosomen treten vermehrt auf Zunahme von Lysosomen in den Mesenchymal aber auch Epithelialzellen deutliche Vakuolisierung des Zytoplasmas 7 500 1

Zellen der RM eine unterschiedliche Funktion der Permeabilitat

Nach der Meinung von Duvall & Wersall (1964) und Musebeck (1963) lokalisiert sich der primare Streptomycineffekt von DHS in den Mitochondrien oder in den Ribosomen (Spoendlin 1966) Da es nach der Streptomycinapplikation in Abhangigkeit von der Dosis und der Anzahl der Injektionen zu einer submikroskopisch nachweisbaren Zerstorung aller membranosen Strukturen der Zellen kommt sind die angefuhrten Moglichkeiten der Ansatzpunkte der Schadigung im Zellstoffwechsel außerordentlich bedeutungsvoll

Die Depression der nuklearen RNS-Synthese und die Abnahme des zytoplasmatischen RNS Gehaltes im Innenohr nach Streptomycinapplikation laßt vermuten daß schon bei relativ niedrigen Dosen zytoplasmatische Ribosomen und chromosomaler Apparat betroffen sind Diese vermutete Beeintrachtigung laßt sich auch fur die Zellorganellen der RM nach Streptomycinapplikation nachweisen

Bei den in der RM an Normaltier histochemisch nachgewiesenen Lipiden handelte es sich durchweg um sogenannte maskierte Fette Mit der Benzpyren Fluoreszenznachweismethode ergab sich fur die RM eine positive Reaktion (Schatzle & Westernhagen, 1967) wie sie auch mit der Sudanschwarz bzw Sudanrot Methode mitgeteilt wurde Diese Befunde konnen jedoch nicht die von uns gefundenen Lipidtropfen erklaren Schätzle (1972) vermutet, daß es sich hierbei um ein durch Streptomycinschadigung hervorgerufenes sekundares Phanomen durch Ansammlung lysosomaler Lipide handelt Von Hawkins (1972) und Ross (1972) wurde vermutet daß es sich hierbei um Lysosomen handele da sich bei der Reaktion auf $Na^+$ offensichtlich eine dieses Gebilde umgebende Membran darstellt Koitschev fand in dieser jedoch keine ATPase



*Abb 6* RM ebenfalls nach 20 Injektionen (s o ) Elektronenoptisch dunkles großes Lysosom auf der mesenthelialen Seite einer Epithel alzelle 28 000 1

Herrn Prof Dr J A Vinnikov und Frau Prof Dr L K Titova mochte ich fur die Unterstützung und Forderung bei der Durchfuhrung dieser Arbeit meinen herzlichen Dank sagen

## SUMMARY

Intense activity was observed in both epithelial and in mesenthelial cells, and, in addition to disturbance of normal activity, destruction of cells from the mesenthelial wall could also be detected It remains to be seen whether, according to the duration of test and the antibiotic used, as well as the dosage, Reissner's membrane is destroyed along a gradient from the peri to the endolymph side, since the pinocytosis derives from the basal membrane and leads to an evident vacuolization of the mesenthelial cells In the same way, the changes within the zona occludens and in the desmosomes are also of importance

## LITERATUR


Ciges, M , Quesada, P & Gonzales, M 1972 Supravital study of guinea pig cochlea referring especially to Kanamycin Labyrinthotoxy *Acta Otolaryng* (Stockh ) *73* 270

Duvall A J & Rhodes, V T 1967 Reissner's membrane An ultrastructural study *Arch Otolaryng* (Chic ) *86* 37

Duvall A J & Sutherland, C. R 1970 The ultrastructure of the extrasensory cells in the cochlear duct In *Symposium on Biochemical Mechanisms in Hearing and Deafness* p 149 Charles C Thomas Springfield Ill

Duvall A J , Sutherland C R & Rhodes, V R 1969 Ultrastructural changes in the cochlear duct following mechanical disruption of the organ of Corti *Ann Otol 78* 342.

— 1972 Pers Mitteilung

Farquhar M G & Palade, G E 1963 Junctional complexes in various epithelia *J Cell Biol 17* 375

Hagiwara S 1963 Electron microscope study on the vestibular membrane (Reissner) *Arch Histol Jap 24* 187

Hawkins J E Jr 1970 Biochemical aspects of ototoxicity In *Symposium on Biochemical Mechanisms in Hearing and Deafness* p 323 Charles C Thomas Publisher, Springfield Ill

Duvall A J & Wersall J 1964 Site of a clion of streptomycin upon inner ear sensory cells *Acta Otolaryng* (Stockh ) *57*, 581

Iurato S 1967 Elektronenoptische Struktur der Innenohr Membranen mit Ruckschlussen auf ihre Eignung zum Stoffaustausch *Arch Ohr Nas Kehl kopfheilk 189* 113

— 1967 *Submicroscopic structure of the inner ear* Pergamon Press Oxford

Iurato S & Taidelli G 1971 The structure of the membrane of Reissner II Internat Symp Gegenwartiger Stand der Cochleaforschung *Wiss Z Univ Halle* XX'71 M, H 1, 105

Kaneko, Y , Nakagawa T & Tanaka, K 1970 Reissner's membrane after Kanamycin administration *Arch Otolaryng* (Chic ) *92*, 457

Kohonen, A 1965 Effect of some ototoxic drugs upon the pattern and innervation of cochlear sensory cells in the guinea pig *Acta Otolaryng* (Stockh ), Suppl *208* 1

Koitschev, K 1972 Pers Mitteilung

Misrahy, G A , Spradley, J F , Dzinovic, S & Brooks, C. J 1962 Effects of intense sound, hypoxia and kanamycin on the permeability of the cochlear portitions *Ann Otol 70*, 572

Musebeck, K 1963 Zum Wirkungsmechanismus der Streptomycin Vergiftung des Ohres *Arch Ohr Nas Kehlkopfheilk 182*, 583

Musebeck, K & Schatzle, W 1962 Experimentelle Studien zur Ototoxicitat des Dihydrostreptomycins *Arch Ohr Nas Kehlkopfheilk 181*, 41

Robertson, J D 1959 The ultrastructure of cell membranes and their derivatives *Biochem Soc Symp 16*, 3

Ross, M D 1972 Pers Mitteilung

Schatzle, W 1972 Pers Mitteilung

Schatzle, W & Westernhagen, B 1967 Lipidnachweis in der Cochlea *Pract Otorhinolaryng* (Basel) 29 73

Spoendlin H H 1966 Die Streptomycinintoxikation beim Meerschwein *Arch Klin Exp Ohr Nas Kehlkopfheilk 194* 562

Spoendlin, H H , Babel, J & Bischoff, A 1971 *Ultrastructure of the peripheral nervous system and sense organs* Georg Thieme, Stuttgart

Stupp, H 1971 Pharmakologische Untersuchungen am Innenohr II Intern Symp Gegenwartiger Stand der Cochleaforschung *Wiss Z Univ Halle* XX'71 M, H 1, 157

Vinnikov, J A & Titova, L K 1961 *Corti Organ Histophysiologie und Histochemie* Ausgabe der Akademie der Wissenschaften der UdSSR, Moskau, Leningrad

— 1964 *The organ of Corti Its histophysiology and histochemistry* Consultants Bureau New York

Watanuki, K 1968 Some morphological observations of Reissner's membrane *Acta Otolaryng* (Stockh ) *66* 40

Watanuki K , Kaseiwacaki H , Kawamoto, K & Katagiri S 1968 The effect of kanamycin intoxication on the RNA metabolism in the cells of Reissner s membrane *Arch Klin Exp Ohr Nas Kehl kopfheilk 192* 375

Watanuki, K , Kawamoto K & Katagiri S 1968 Some autoradiographic observations of the nucleic acid metabolism in the cells of Reissner s membrane *Arch Klin Exp Ohr Nas Kelhkopfheilk 192* 91


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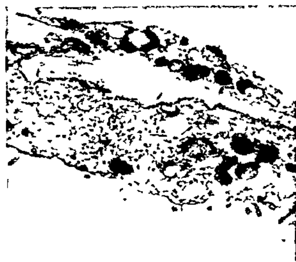


*Abb 5* RM ebenfalls nach 20 Injektionen (s o ) Freie Ribosomen treten vermehrt auf, Zunahme von Lysosomen in den Mesenchymal aber auch Epithelialzel len deutliche Vakuolisierung des Zytoplasmas 7 500 1

Zellen der RM eine unterschiedliche Funktion der Permeabilitat

Nach der Meinung von Duvall & Wersall (1964) und Musebeck (1963) lokalisiert sich der *primare Streptomycineffekt von DHS in* den Mitochondrien oder in den Ribosomen (Spoendlin, 1966) Da es nach der Streptomycinapplikation in Abhangigkeit von der Dosis und der Anzahl der Injektionen zu einer submikroskopisch nachweisbaren Zerstorung aller membranosen Strukturen der Zellen kommt, sind die angefuhrten Moglichkeiten der Ansatzpunkte der Schadigung im Zellstoffwechsel außerordentlich bedeutungsvoll

Die Depression der nuklearen RNS-Synthese und die Abnahme des zytoplasmatischen RNS-Gehaltes im Innenohr nach Streptomycinapplikation laßt vermuten daß schon bei relativ niedrigen Dosen zytoplasmatische Ribo somen und chromosomaler Apparat betroffen sind Diese vermutete Beeintrachtigung laßt sich auch fur die Zellorganellen der RM nach Streptomycinapplikation nachweisen

Bei den in der RM an Normaltier histochemisch nachgewiesenen Lipiden handelte es sich durchweg um sogenannte maskierte Fet te Mit der Benzpyren Fluoreszenznachweis methode ergab sich fur die RM eine positive Reaktion (*Schatzle & Westernhagen*, 1967) wie sie auch mit der Sudanschwarz- bzw Sudanrot-Methode mitgeteilt wurde Diese Befunde konnen jedoch nicht die von uns gefundenen Lipidtropfen erklaren Schatzle (1972) vermutet, daß es sich hierbei um ein durch Streptomycinschadigung hervorgerufenes sekundares Phanomen durch Ansammlung lysosomaler Lipide handelt Von Hawkins (1972) und Ross (1972) wurde vermutet, daß es sich hierbei um Lysosomen handele, da sich bei der Reaktion auf $Na^{+}$ offensichtlich eine dieses Gebilde umgebende Membran darstellt Koitschev fand in dieser jedoch keine ATPase



*Abb 6* RM ebenfalls nach 20 Injektionen (s o ) Elektronenoptisch dunkles, großes Lysosom auf der me senthelialen Seite einer Epithelialzelle 28 000 1

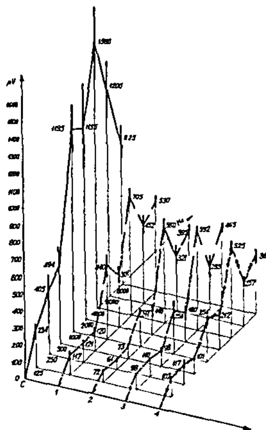


Fig 1 Curve of CM values after 12 weeks in μV in the various groups of animals *C* Control group *1* Group exposed to noise *2* Group given kanamycin for 1 week before 12 weeks exposure to noise *3* 2 weeks kanamycin before 12 weeks exposure to noise *4* 2 weeks kanamycin during 12 weeks exposure to noise Acoustic stimulus = 80 dB

The Preyer reflex was tested in all the animals before the experiments were begun and animals with a weak reflex were not included in the experiments

## RESULTS

The mean values of the CM and AP voltages were determined the standard deviations calculated and a statistical analysis of the results was made on the basis of the unilateral Dunnet test The mean from the control group was taken as the standard in the Dunnet test and the results obtained from the other groups were analysed in relation to this standard $\alpha - 0\,05$ was taken to be the significance level The calculations were made on a computer Odra 1204 at the Electronic Computation Technique Centre of the Institute of Energetics Warsaw Most of the findings were statistically significant

In all the groups of animals the changes in CM voltage were found to be phasic In group I the animals exposed to noise only the greatest percentage fall in CM voltage was observed 1 week after this exposure and a similarly large fall in CM continued up to 2 weeks of exposure to noise This fall was noted at all frequencies but the greatest occurred at 2 000 Hz (77%) and 6 000 Hz (84%) After the 3rd and 4th week a gradual fall in CM was observed in the low and medium frequency ranges whereas the CM voltage at high frequencies (from 4 000–8 000 Hz) rose A period of 6–12 weeks exposure to noise caused a gradual rise in CM at all frequencies The CM voltage after 12 weeks exposure exceeded that of the CM voltage noted after 2 weeks When an acoustic stimulus of 80 dB was applied the greatest fall in CM was observed at frequencies of 2 000 Hz (74%) and 500 Hz (76%) With a stimulus of 60 dB however the fall in CM occurred at 2 000 Hz (77%) and at high frequencies 6 000 Hz (74%) and 8 000 Hz (71%)

The dynamics of the CM changes in animal group II to which kanamycin was administered 1 week before exposure to noise were similar the only difference being that after 12 weeks exposure a marked fall in CM occurred at all frequencies the greatest percentage fall occurred at 2 000 Hz (87%) with an acoustic stimulus of 80 dB and when 60 dB were applied the fall occurred at high frequencies 6 000 Hz (91%) 8 000 (88%) and at a frequency of 2 000 Hz (89%)

In group III which received kanamycin 2 weeks before exposure to noise the first phase of the fall in CM was prolonged continuing up to the end of the 3rd week After 4 weeks a gradual rise in CM was observed beginning

with the high frequencies and, after 6 weeks, occurring at all frequencies After 12 weeks, however, a marked fall in CM at all frequencies was noted The greatest percentage fall occurred with an 80 dB stimulus at 2 000 Hz (84%) and with an intensity of 60 dB this fall was noted at the high frequencies, 6 000 Hz (88%), 8 000 Hz (82%), and at 2 000 Hz (85%)

The first phase of the CM fall continued for 3 weeks in the group IV which received kanamycin for 2 weeks while being exposed to noise The greatest percentage fall occurred when both these factors, noise and kanamycin, affected the hearing simultaneously The CM rose at all frequencies after 4 weeks, but after 6 weeks, i e earlier than in the previous groups, another depression of CM occurred which deepened in the 12th week When tested with tones of 80 dB, the greatest percentage fall was noted at 2 000 Hz

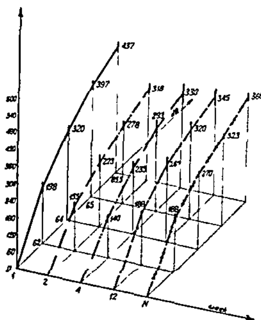


*Fig 3* Curve of AP values in μV in group I in comparison with control group *N* Control group After exposure to noise for 1 week (*1*) 2 weeks (*2*) 4 weeks (*4*) 12 weeks (*12*)

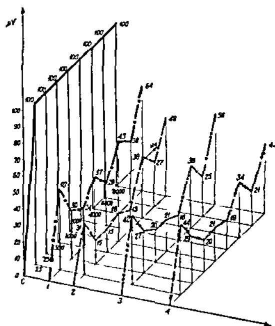


*Fig 2* Curve of CM values per cent after 12 weeks in the various groups of animals *C* Control group *1* Group exposed to noise *2* Group given kanamycin for 1 week before 12 weeks exposure to noise *3* 2 weeks kanamycin before 12 weeks exposure to noise *4* 2 weeks kanamycin during 12 weeks exposure to noise Acoustic stimulus = 80 dB

(81%) and with tones of 60 dB at 6 000 Hz (84%), 8 000 Hz (84%) and 2 000 Hz (81%)

Together with these tests, an analysis of the AP values noted in the various groups was carried out The analysis was based on comparison of the $N_1$ AP amplitude after 1, 2, 4, and 12 weeks In groups I, II, and III, the changes in AP voltage were analogical After 1 weeks' exposure to noise, the AP voltages rose to a level higher than that of the control group After 2 weeks, the AP fell below that of the control group After 4 weeks there was no tendency to a fall in AP After 12 weeks, the AP values equalled those of the controls in group I, whereas the AP values of group II and III were slightly higher than the control values

The dynamics of the AP changes in group IV, animals which were given kanamycin while being exposed to noise, differed somewhat The first phase, during which the AP values exceeded those of the controls lasted for a whole 2 weeks and the second phase

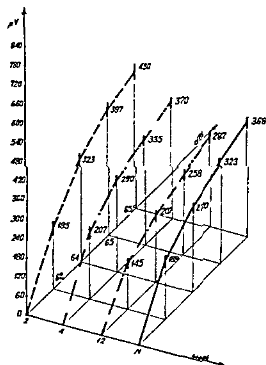


*Fig 4* Curve of AP values in μV in group IV N Control group After exposure to noise for 2 weeks (2) 4 weeks (4) 12 weeks (12)

that of a fall in AP values, lasted to the end of the 12th week

The peripheral adaptation was also studied by comparing the values of unadapted $N_1$ AP by increasing the frequency of the stimulus (100, 200, 400, 500 clicks/sec)

In all the groups of animals, their adaptive capacity was retained, its increase being a function of the stimulus frequency In all the experimental groups, the adaptation was less than that of the controls The adaptation of the controls was found to be 22%–34%, that of group I, 10%–15%, of group II 12%–17%, of group III, 13%–19%, of group IV, 15%–21% No marked regularity of adaptation in relation to the period of exposure to noise was noted (the results were not statistically significant) There was only a greater variation in adaptation in the groups of animals subjected to both noise and kanamycin

## DISCUSSION

Most authors have observed a fall in CM and AP when the animal is exposed to noise (Gisselsson & Sørensen, 1959, Lawrence et al, 1959, Ruedi & Furrer, 1947, Sekuła & Trąbka, 1967) but the dynamics of the CM and AP changes over a longer period of time have not so far been published Jankowski et al (1971) observed the dynamics of CM and AP changes over a period of 1–6 days

As has been reported by other authors (Hawkins, 1959, Jankowski et al, 1971, Lawrence et al, 1959) and as was found in our investigations, the fall in CM occurs at all frequencies In our investigations the extent of the fall in CM voltage was greater when an acoustic stimulus of a lower intensity, 60 dB, was applied (this is in agreement with the findings of Wagner & Gerhardt, 1963) and it was noted that the fall in CM occurs above all at the high frequencies (similar results were reported by Jankowski et al., 1971) and at a frequency of 2 000 Hz, as was observed by Lawrence et al (1959)

The dynamics of the changes in CM had a marked phasic character regardless of the intensity of the acoustic stimuli The first phase, lasting up to 2 weeks inclusive, was characterized by the greatest percentage fall During the second phase, which lasted 4 weeks, a certain stabilization occurred, characterized by a minimal fall in CM at low and medium frequencies and a rise in CM at high frequencies The third phase, of 12 weeks' duration, brought a further restitution of CM at all frequencies In the animals which received kanamycin before being exposed to noise and while being exposed to noise, a fourth phase was observed, that of another fall in PM voltage This phase occurs earlier (after 6 weeks' exposure) in the animals which received kanamycin while being exposed to noise than in those animals which were given kanamycin before exposure (after 12 weeks)

On the basis of extensive comparative studies, Wever (1966) found that there is a marked

In all the groups of animals, the peripheral adaptation which was preserved, though lower than that of the control group, is, according to Rosenblith (1954) and Stange et al (1964) evidence of deterioration in the function of the transformation organ

## CONCLUSIONS

1 The dynamics of the electrocochleogram changes (CM and AP) in the guinea pig while under the influence of industrial noise, and kanamycin + industrial noise are phasic

2 The phase of the changes in CM and AP differs

3 The phases of the changes in CM in the animals subjected to industrial noise for 12 weeks consisted of a phase of fall, a phase of stabilization and a phase of regression When the animals were exposed to two harmful agents another phase of a secondary fall occurred

4 The changes in AP were characterized by periods of rise, fall, and regression, in the animals which were exposed to both noise and kanamycin at the same time, the phases of rise and fall were prolonged

5 The extent of the fall in CM depends on the intensity of the acoustic stimulus used for the investigations

6 In the regression phase, the rise in CM voltages begins at the high frequencies

7 Peripheral adaptation to industrial noise and kanamycin deteriorates

8 The findings confirmed by statistical methods indicate that kanamycin predisposes the ear to damage by industrial noise, particularly when this drug is administered during exposure to such noise

## ZUSAMMENFASSUNG

Bei 250 wahrend einer bis zu 12 Wochen mit Industrielarm sowie Kanamyzin und Larm belasteten Meerschweinchen wurde die Veranderungsdynamik des Elektrocochleograms (CM AP) untersucht Es wurde eine Phasendynamik der CM und AP Veranderungen sowie eine bewahrte aber herabgesetzte periphenische Adaptation festgestellt Die erzielten statistisch bestatigten Ergebnisse weisen auf einen pradisponieren den Einfluss des Medikaments mit ototoxischer Nebenwirkung auf die Beschleunigung der industriellen Beschadigung des Gehors hin und auf eine besonders schnelle Wirkung bei Verabreichung des Mittels wahrend der Larmbelastung

## REFERENCES

Beck Ch 1971 Die Bedeutung vergleichender Untersuchungen am akustischen System *Acta Otolaryng* (Stockh ) *71* 206

Davis H Deatherage B H Rosenbluth B Fernandez C Kimura R & Smith C A 1958 Modification of cochlear potentials produced by streptomycin poisoning and by extensive venous obstruction *Laryngoscope 68* 596

Dieroff H G 1963 *Die Larmschwerhorigkeit in der Industrie* J A Barth Leipzig

Engstrom H 1967 The morphology of the normal sensory cell *Acta Otolaryng* (Stockh ) *63*

Engstrom H & Kohonen A 1965 Cochlear damage from ototoxic antibiotics *Acta Otolaryng* (Stockh ) *59* 171

Farkashidy J Balck R G & Briand T D R 1963 The effect of kanamycin on the internal ear An electrophysiolog cal and electron microscopic study *Laryngoscope 73* 713

Gisselsson L & Soerensen H 1959 Auditory adaptation and fatigue in cochlear potentials *Acta Otolaryng* (Stockh ) *50* 391

Hassmann W Filipowski M Krochmalska E Pietruski J & Korn Rydzewska H 1970 Zawodowe uszkodzenie słuchu u pracowników przemyslu włokienniczego *Pamiętnik XXVII Zjazdu Otolaryngologów Polskich* PZWL Warszawa 130

Hawkins J E 1959 The ototoxicity of kanamycin *Ann Otol 68* 698

Hawkins J E & Engstrom H 1964 Effect of kanamycin in cochlear cytoarchitecture *Acta Otolaryng* (Stockh ) Suppl *188* 100

Jankowski W & Ziemski Z 1968 Działanie osłaniające cytochromu C w miejscowym zatruciu kanamycyną lub streptomycyną narządu Cortiego zwierzęcia *Otolaryng Pol 22* 513

Jankowski W Ziemski Z Birecki W Kusek H Cyrulewska Orłowska J , Praga J & Kowalewska M 1971 Wpływ hałasu przemysłowego na zachowanie się biopotencjałów ucha wewnętrznego świnki morskiej *Otolaryng Pol 25* 267

Lawrence M Wolsk D & Burton R 1959 Stimulation deafness cochlear patterns and significance of electrical recording methods *Ann Otol 68* 1

Merkle U Plattig K H & Keidel U O 1968 Histologische Untersuchungen zur ototoxischen Wirkung des Kanamycins am Cortischen Organ der Katze *Z Mikroskopisch Anat Forsch 78* 441

Rosenblith W A 1954 Electrical responses from the auditory nervous system *Ann Otol 63* 840

Rued L & Furrer W 1947 *Das akustische Trauma* S Karger Basel

Sekula J & Trąbka J 1967 Elektryczne potencjały czynnosciowe analizatora sluchowego *Acta Physiol Pol 18* 747

Spoendl n H 1969 Innervation patterns in the organ of Corti *Acta Otolaryng* (Stockh ) *67* 239

Stange G Spreng M & Keidel U O 1964 Die Wirkung von Streptomycinsulfat auf Erregung und Adaptation der Haarzellen des Cortischen Organs *Pfluger Arch Ges Phys ol 279* 99

Tyberghein J 1962 Influence of some streptomyces antibiotics on the cochlear microphonics in the guinea pig *Acta Otolaryng* (Stockh ) Suppl *171*

Wagner H & Gerhardt H 1963 D e Wirkung dos er ter Reintonbelastung und d e Mikrophonpotentiale der Meerschweinchenschnecke *Arch Ohr Nas Kehl Kopfheilk 181* 82

Wever E G 1966 Electrical potentials of the cochlea *Physiol Rev 46* 102

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*Poland*

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# TEMPORARY HEARING LOSSES IN TEEN-AGERS ATTENDING REPEATED ROCK-AND ROLL SESSIONS

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*Abstract* This study investigated the hearing of teen agers who voluntarily exposed themselves to repeated sessions of loudly amplified pop music Hearing thresholds were measured before and 30 min after ex posure for each of 8 weekly sessions of rock-and roll music with an average sound pressure level of 110 dB to 115 dB Significant temporary threshold shifts were found in all subjects especially in the high frequencies Hearing sensitivity recovered between the repeated exposures and a 5 month follow up study found that the hearing in all subjects but one had returned to its initial pre-exposure level The exposure had dif ferential effects on the two ears at the same test frequencies

Increasing concern about environmental noise pollution has prompted studies other than those in which acoustic trauma has been experimen tally induced or attributed to industrial or military exposures to loud sounds In the past decade the majority of our young people have voluntarily exposed themselves to highly am plified rock and roll, discothèque, or pop mu sic This type of music has been investigated from several different points of view Meas-urement of sound pressure levels and frequency distributions led Lebo & Oliphant (1968) to conclude that prolonged exposure to such music was unsafe Kryter (1970) described rock and roll as intermediate between impulsive and non impulsive sound and pointed out that it exceeded limits set forth in damage risk cri teria Rintelmann & Borus (1968) investigated musicians who were exposed to this type of music as an occupational hazard and reported that only 5% of 42 performers had permanent hearing losses after long term exposure Most workers have agreed that rock and roll pro-duced at least temporary changes in the thresh old of hearing Rice et al (1968), Speaks et al (1970) and Jerger & Jerger (1970) meas ured shifts in the hearing thresholds of per-formers at 2 min, 20 to 40 min, and 1 hour respectively after exposure Temporary hear-ing losses were present in almost all subjects, and permanent losses were recorded in about 25% of the musicians Laboratory studies be cause of the recognized risk to hearing have necessarily limited the exposure of subjects to rock and roll music to one session of an hour or less (Dey, 1970, Hickling 1970) Re cently Rintelmann et al (1972) had 20 sub-jects listen to an hour of rock and roll music under both continuous and intermittent con ditions The hearing recovery pattern was fol

This research was completed while Dr Ulrich was a postdoctoral fellow in the Division of Neurology De partment of Medicine Case Western Reserve Uni versity School of Medicine and University Hospitals of Cleveland Ohio supported by USPHS Grant Num ber 5 T01 NS05087 14 of the National Institute of Neurological Diseases and Stroke National Institute of Health Dr Pinheiro was a postdoctoral fellow in the Department of Speech Communication Case West ern Reserve University and was supported by N I H Grant Number 5 T01 NB05437-08

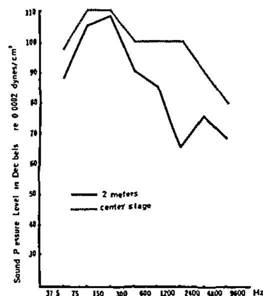



*Fig 1* Average octave band frequency spectrum Data points represent the average measurement for the bandwidth between the two frequencies indicated at either side of each data point

lowed up to 90 min after exposure, and it was concluded that daily exposures over an extended period would be hazardous to hearing

The purpose of the present study was to investigate the temporary and any possible permanent noise induced hearing losses in a group of teen agers actually exposed to long hours of highly amplified live rock and roll music over a series of eight weekly discotheque sessions These sessions were part of a city summer recreational program for young people and were held in an outdoor amphitheater Each session was frequented by well over a thousand teen agers Exposure of human subjects to such loud sound for several hours over repeated sessions is not readily possible in a laboratory setting

Of the randomly selected teen agers who were willing to have their hearing tested before and after each weekly rock and roll session, 14 completed the project These included 4 boys and 10 girls ranging in age from 13 to 17 years None had any history of ear disease, familial hearing loss, or ingestion of oto toxic drugs

Two calibrated audiometers were set up in separate rooms of a quiet basement in a home a short distance from the park where the rock and-roll sessions took place Ambient noise level, measured in the test environment for each session, did not exceed 40 dB sound pressure level (SPL) Two experimenters administered pure tone hearing tests to the subjects before each rock-and-roll session and again 30 min after the session The same tester and the same audiometer were used on each occasion for a given subject Both ears were tested by air conduction at 250, 500, 1 000 2 000, 4 000, and 8 000 Hertz (Hz) for both pre-exposure and post-exposure measurements for the eight weekly sessions Pure tone bone conduction thresholds were measured at the initial pre exposure test and at the follow up hearing evaluation conducted 5 months after the last rock-and-roll program

The sound pressure level and frequency distribution of the rock-and-roll music were measured weekly using the slow setting and both A and C filters of a Rudmose RA-100 Sound Level Analyzer Measurements were taken at different positions around the large stage Fig 1 displays an average of the sound level meter readings for the various frequency bands at 2 meters in front of the performers and at center stage At the farthest edge of the stage the SPLs were about 10 dB less At a distance of the sixth row in the amphitheater, the over all SPL was 90 dB to 95 dB Side and center stage readings had an overall average of 110 dB and 115 dB SPL respectively The loudest range of the spectral distribution was from 75 Hz to 1 200 Hz with a slight peak between 300 Hz and 600 Hz There were times during measurements of the sound levels that the SPL peaked beyond the 150 dB maximum capacity of the meter The rock and roll music was intermittent in that there was about a minute between selections When one group of performers took a rest, a second combo played after an interval of less than 5 minutes Each weekly session lasted for 3 1/4 hours

Table I *Average temporary threshold shift in dB for eight rock and roll sessions*

| 250 Hz | 500 Hz | 1 000 Hz | 2 000 Hz | 4 000 Hz | 8 000 Hz |
|---|---|---|---|---|---|
| *Right ear* | | | | | |
| 7 9 | 4 3 | 1 8 | 10 7 | 18 2 | 16 4 |
| *Left ear* | | | | | |
| 1 1 | 0 0 | 7 1 | 13 9 | 15 4 | 11 1 |

## RESULTS

The 30 min post exposure audiograms showed temporary threshold shifts (TTS) for all sub jects at three or more of the six test frequencies in both ears Table I displays the average threshold shift found for each ear at each frequency Individual subjects varied widely in the amounts of TTS in their post exposure audiograms, but all subjects demonstrated the most TTS at 4 000 Hz with 2 000 Hz and 8 000 Hz affected to a lesser degree

When pure tone test results for before and after the rock-and roll sessions were compared, the *t* scores were statistically significant at the 95% level of confidence for 2 000, 4 000, and 8 000 Hz in both ears Therefore, even after 30 min of recovery time, the amount of temporary hearing loss present at these frequencies was significant

Pure tone hearing thresholds for each subject were highly correlated across pre-exposure measurements, indicating good intra subject reliability Each pre exposure audiogram showed that the subject s hearing sensitivity had recovered from the TTS of the previous week's exposure Subjects who initially had small threshold shifts tended to have less TTS for succeeding rock and roll sessions than subjects who initially had large shifts in hearing levels When post exposure thresholds for the first and last sessions were statistically com pared, the left ear showed a significant in crease in TTS at 4 000 Hz for the last session and a significant decrease in TTS at 500 Hz and 1 000 Hz. The right ear had significantly greater TTS at 1 000 Hz and at 4 000 Hz for the last exposure with an increment in threshold shift apparent at all test frequencies The results are graphically displayed in Fig 2

Five months after the last rock and roll session, pure tone thresholds were again meas ured on all subjects Subjects reported that they had not been exposed to any further discotheque sessions, although most listened to loud radio music at home All subjects had hearing within the normal range with air con duction thresholds equal to bone conduction thresholds When initial tests were compared with the follow-up audiograms, it was evident that the hearing sensitivity of most of the teen age subjects had returned to original lev els within ±5 dB One subject, however, had his hearing threshold diminished by 10 dB to 15 dB at four frequencies in the left ear and at one frequency in the right ear This particular subject was not one of the several subjects who showed large TTS on post-exposure tests

## DISCUSSION AND CONCLUSION

Results of this study closely agree with other reports in the literature on TTS due to ex posure to rock and roll Although this project forfeited the more rigorous control of laboratory experiments in exchange for subjects who voluntarily exposed themselves to highly am plified pop music for several hours in repeated sessions, the TTS found for individuals was similar to that published by Jerger & Jerger (1970) for two groups of performers tested one hour after exposure Average TTS in this study was slightly greater than Rintelmann

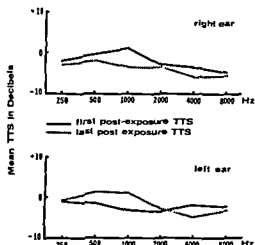



*Fig 2* Comparison of post-exposure TTS for first and last rock and roll sessions The 0 line represents pre exposure threshold Deviation above 0 represents improved sensitivity Deviation below 0 indicates de creased sensitivity

and his co-workers (1972) measured at 30 min post exposure to intermittent rock-and-roll music However, Rintelmann's subjects were exposed for only one hour

Sound pressure levels measured during the rock-and-roll sessions differed little from those eported by other investigators for numerous p groups (Lebo & Oliphant, 1968, Rintel-ann & Borus 1968, Speaks & Nelson, 1968, Lipscomb, 1969) Since the sessions in this study were held in the open air, one might have *expected that TTS would have been less* than that previously reported However, this was not the case

Of particular interest were the differences in TTS between the two ears at the same frequencies Jerger's (1970) study showed simi lar differential effects in the TTS in the two ears of the performers Ward (1967) pointed out that the same ear may also exhibit different susceptibilities to different frequency bands In this study the average TTS was greater at 250 Hz and 500 Hz in the right ear The left ear had more TTS than the right ear at 1 000 Hz and 2 000 Hz, and the right ear had more TTS at 4 000 Hz and 8 000 Hz than the left ear It is difficult to determine the reason for such differences in susceptibility One might hypothesize that the micro*scopic physical variations between the two ears* in the position or angle of the cochlear duct relative to the oval window could be responsible Such a difference might cause the fluid pressure waves in the inner ear to stress the sensory structures at slightly different points

The fact that this project followed the subjects through a series of weekly exposures to rock-and roll permitted a comparison of TTS between initial and final sessions Although individual hearing recovered between sessions, the average TTS generally became worse over the succession of weekly exposures While the mean right ear TTS was greater for the final exposure at all frequencies, left ear TTS for the final session was greater than after the first session only at frequencies above 2 000 *Hz Part of the explanation for this result* might be that the left ear suffered more ini tial TTS than the right ear in the lower fre quencies The smaller TTS after the final exposure might indicate that the inner ear had already undergone physiological changes so that lateral inhibition along the cochlear partition was affected, making hearing appear more sensitive to clinical testing The mean left ear TTS at 1 000 Hz was initially greater than that of the right ear at the same frequency With the higher frequencies in the mid lower *basal turn of the cochlea greatly depressed,* the inner ear response to lower frequencies farther along the basilar membrane might have been protected or less inhibited by lateral inhibition

Although the relationship between noise-induced temporary and permanent threshold shifts is still not clear, the fact that the hearing of most teen-agers in this study returned to original levels of sensitivity does not rule out possible damage to the sensory elements of the inner ear Histological examinations of both human and animal ears have shown that large numbers of hair cells may be missing along the organ of Corti when hearing thresholds are reported to be clinically normal Re-

cent data from studies of the cochleae of chinchillas and monkeys exposed to loud noises to produce TTS, revealed that the inner ear underwent observable permanent changes in spite of the recovery of normal hearing sensitivity (Mills et al, 1970 Miller et al, 1971, Pinheiro et al, 1972)

There was no way to determine whether the permanent decrease in hearing sensitivity found at follow up in one of the subjects was due to the repeated rock and roll exposures Although no other explanation could be found there was no difference greater than 5 dB between his initial audiogram and his last pre exposure hearing test

## SUMMARY

The hearing sensitivity of teen age subjects exposed to several hours of rock and roll music in repeated weekly sessions recovered to pre exposure levels in spite of significant post exposure threshold shifts TTS for the two ears differed for the same frequencies There was generally greater TTS for the final exposure than for the initial exposure Research suggests that recovery of clinically normal hearing after TTS does not preclude permanent damage to cochlear sensory structures

## ZUSAMMENFASSUNG

Diese Untersuchung befasst sich mit dem Gehororganismus von Teenagers die sich freiwillig des often zum Anhoren von lautverstarktem Rock and Roll zur Verfugung gestellt haben Vor Beginn und 30 Minuten nach jeder der 8 wochentlichen Musikexperimente d e eine durchschnittl che Lautstarke von 110 dB bis 115 dB hatten wurde der Horfrequenzbereich gemessen Bedeutende vorubergehende Veranderungen im Horfrequenzbereich wurden bei allen Versuchspersonen festgestellt hauptsachlich in den hohen Frequenzen Die Gehorsensitivitat erholte s ch zwischen den wiederholten Experimenten und eine Nachuntersuchung nach 5 Monaten ergab dass das Gehor aller mit einer Ausnahme auf den Zustand vor dem Ganz experiment zuruckgegangen war Verschiedene Effekte auf beide Ohren in derselben Frequenz wurden fest gestellt

## REFERENCES

Dey F I 1970 Auditory fatigue and predicted permanent hearing defects from rock and roll music *New Engl J Med 282* 467

Hickl ng S 1970 Noise induced hearing loss and pop music *New Zeal Med J 71* 94

Jerger J & Jerger S 1970 Temporary threshold shift in rock and roll musicians *J Speech Hearing Res 13* 221

Kryter K D 1970 *The effects of noise on man* Academic Press New York

Lebo C P & Oliphant K P 1968 Music as a source of acoustic trauma *Laryngoscope 78* 1211

Lipscomb D M 1969 High intensity sounds in the recreational environment *Clin Pediat* (Bologna) *8* 63

Miller J D Rothenberg S J & Eldredge D H 1971 Preliminary observat ons on the effects of exposure to noise for seven days on the hearing and inner ear of the chinchilla *J Acoust Soc Amer 50* 1199

Mills J H Gengel R W Watson C S & Miller J D 1970 Temporary changes of the auditory system due to exposure to noise for one or two days *J Acoust Soc Amer 48* 524

Pinheiro M L Jordan V M & Luz G A 1972 The relationship between permanent threshold shift and the loss of hair cells in monkeys exposed to impulse noise *USAMRL Report No 990*

Rice C G Ayley J B Bartlett B Bedford W Gregory H & Hallum G 1968 A pilot study on *the effects of pop group music on hearing I S V R Memo 226* Univers ty of Southampton Press Southampton Eng

Rintelmann W F & Borus J F 1968 Noise-induced hearing loss and rock and roll music *Arch Otolaryng* (Chic ) *88* 377

Rintelmann W F Lindberg R F & Smitley E K. 1972. Temporary threshold shift and recovery patterns from two types of rock and roll music presentation *J Acoust Soc Amer 51* 1249

Speaks C & Nelson D 1968 TTS following exposure to rock-and roll music Paper presented at the 76th meeting of the Acoustical Society of America Cleveland Ohio

Speaks C. Nelson D & Ward W D 1970 Hearing loss in rock and roll musicians *J Occup Med 12* 216

Ward W D 1967 Susceptibility to auditory fatigue *Advances in sensory physiology* (ed W D Neff) Academic Press New York

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# ACTION OF CHOLINERGIC AND ANTICHOLINERGIC DRUGS AT THE CROSSED OLIVOCOCHLEAR BUNDLE – HAIR CELL JUNCTION

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*Abstract* Both cholinergic and anticholinergic drugs introduced into the scala tympani of guinea pigs were found to block the effects induced by electrical stimulation of the crossed olivocochlear bundle Strychnine d tubocurare and decamethonium were most potent in this activity Eserine did not potentiate the effects of electrical stimulation of the crossed olivocochlear bundle but did potentiate the effects of acetylcholine and in high concentrations blocked the crossed olivocochlear bundle Acetylcholine (247 $\mu$M) in combination with eserine (20 $\mu$M) mimicked the effects of the crossed olivocochlear bundle by producing an augmentation of the cochlear microphonics and an inhibition of the action potential The acetylcholine (247 $\mu$M)-eserine (20 $\mu$M) combination also produced a reversible blockade of the crossed olivocochlear bundle and the mimicking effects of subsequent perfusions with the drug combination The mimicking effect of the acetylcholine (247 $\mu$M)-eserine (20 $\mu$M) combination was blocked by d tubocurare *These results further extend the evidence which has accumulated towards proving that acetylcholine is the transmitter substance of the crossed olivocochlear bundle*

The crossed olivocochlear bundle is an efferent inhibitory tract of nerve fibers which originate in the accessory nucleus of the superior olivary complex and form synaptic junctions at the base of the hair cells of the contralateral cochlea (Smith, 1967) Electrical stimulation of these fibers results in an inhibition of the compound action potential of the auditory nerve (Galambos, 1956), an augmentation of the cochlear microphonics (Fex, 1959), an increase in the positive summating potential (Konishi & Kelsey, 1970), and a slow negative change in the endocochlear potential (Fex, 1967 *a*) Cholinesterase (Schuknecht et al, 1959) and nerve endings containing synaptic vesicles (Smith, 1967) have been associated with these efferent fibers in the cochlea Therefore, it seems that a transmitter substance may be released by the crossed olivocochlear bundle nerve endings

Many laboratories have tested the hypothesis that acetylcholine or gamma aminobutyric acid is the inhibitory transmitter substance (as cited in Guth & Bobbin 1971) Conflicting results were obtained when iontophoretic drug application was used (Gisselsson, 1960 Tanaka & Katsuki, 1966) Other pharmacological studies appear to have been hampered by a blood cochlear barrier which prevented drugs from reaching the site of the efferent nerve endings Thus cholinergic agents applied intra arterially evoked an inhibition of the action potential (Brown et al, 1969, Amaro et al, 1966), a result which is similar to that resulting from electrical stimulation of the crossed olivocochlear bundle Anticholinergics applied in the same manner prevented the drug induced response (Amaro et al, 1966 Daigneault & Brown 1966) but

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did not prevent the crossed olivocochlear bundle induced response (Guth & Amaro, 1969, Brown et al, 1969)

The technique of placing test solutions containing *d* tubocurare in perilymphatic space of the scala tympani has been shown to block the cochlear responses to electrical stimulation of the efferent nerve fibers (Fex, 1968, Konishi, 1972) Thus, the intracochlear application of drugs appears to by-pass vascular barriers to the nerve-hair cell junctions Therefore, this study was designed to test whether acetylcholine is the transmitter substance of the crossed olivocochlear bundle through the use of the scala tympani perfusion technique to determine whether acetylcholine duplicates the effects of electrical stimulation of the crossed olivocochlear bundle, the influence of known acetylcholine antagonists on the effects of electrical stimulation of the crossed olivocochlear bundle, and the influence of eserine, an inhibitor of cholinesterase, on both the acetylcholine induced and crossed olivocochlear bundle induced effects A preliminary report of the data presented herein has been published (Bobbin & Konishi, 1971)

## METHODS

Pigmented guinea pigs (200–300 g) of either sex were used They were anesthetized with Sodium Pentobarbital (Nembutal®, Abbott, 25–30 mg/kg i p) The jugular vein was cannulated for intravenous drug administration The trachea was also cannulated In all animals the right auditory bulla was exposed and opened The middle ear muscle tendons were sectioned in most animals After this surgical procedure the animals were placed in an acoustical and electrical shielded room Gallamine triethiodide (Flaxedil®, Davis & Geck, 8 mg/kg, i v) was administered and the animal maintained by artificial respiration to prevent movements of the animal

The differential electrode technique was employed at the basal turn of the cochlea to record sound evoked responses (Tasaki et al, 1952) Tone bursts of 6 kHz and 600 Hz with durations of 10 msec and rise and fall times of 0 5 msec were delivered to the external auditory canal through a closed system Both the action potential and cochlear microphonics were recorded in response to the 6 kHz tone burst whereas only the cochlear microphonics were recorded in response to the 600 Hz tone burst

The endocochlear potential was recorded by a microcapillary electrode filled with 3 M KCl which was inserted into the scala media through the spiral ligament (Konishi et al, 1961) A chloride-coated silver wire placed on the intact neck muscles served as the reference electrode The cerebellum was exposed and a part of it was suctioned away to uncover the floor of the fourth ventricle Bipolar stimulating electrodes were placed under visual control between the facial genua on the floor of the fourth ventricle in order to stimulate the crossed olivocochlear bundle The electrode was placed along the midline of the brain stem where the largest negative change in the endocochlear potential could be recorded This negative change in the endocochlear potential (the crossed olivocochlear potential) has been described (Fex, 1967, 1967 *a*, Konishi & Kelsey, 1970) The electrical stimulus was a train of impulses (pulse duration, 0 3 msec, pulse frequency, 400/sec, train duration, 100 msec, current, 0 08–0 20 mA) which preceded the tone bursts by 10 msec

The cochlear potentials including the endocochlear potential were displayed on an oscilloscope and photographed by a Grass $C_4$ kymograph camera The endocochlear potential was also recorded on a Beckman Type R chart recorder

The methods used to perfuse the scala tympani with an artificial perilymph have also been cited and described elsewhere (Konishi & Kelsey, 1970) The artificial perilymph used was composed of NaCl 139 mM, KCl, 5 6 mM, $CaCl_2$, 2 2 mM, $NaHCO_3$, 21 mM, gassed

Table I *Effect of perfusion of the scala tympani with artificial perilymph and acetylcholine (247 μM) together with eserine (20 μM) on the action potential (AP) and the cochlear microphonics (CM)*

| | No of animals | AP 6 kHz (%)[a] | CM 6 kHz (%) | CM 600 Hz (%) |
|---|---|---|---|---|
| Artificial perilymph | 12 | −18±3 | −2±1 | +9±2 |
| Acetylcholine–eserine | 4 | −54±3 | +24±2 | +64±5 |

[a] Results are presented as the mean percentage change from control together with the standard error The data are taken from the first perfusion in each animal

with 95% $O_2$ 5% $CO_2$, with resultant pH of 7 2 Drug concentrations in the artificial perilymph are expressed in terms of the free base The perfusion solutions were applied to the scala tympani at room temperature (approximately 25°C) The rate of perfusion was slow (2 μl/min) so as to minimize any displacements of cochlear tissues A constant duration of perfusion (10 min) was used Before each perfusion several control observations were made over a period of at least 10 min to insure that all cochlear responses were stable

The following drugs were studied carbamylcholine chloride (carbachol), acetylcholine chloride, eserine sulfate, choline iodide, nicotine tartrate, *d* tubocurarine chloride, strychnine sulfate, atropine sulfate (atropine), atropine methyl bromide (methylatropine), arecoline hydrobromide hexamethonium chloride, and decamethonium bromide

## RESULTS

### *Action of artificial perilymph*

The first perfusion of the scala tympani with artificial perilymph suppressed the action potential and the cochlear microphonics in response to 6 kHz and augmented the cochlear microphonics in response to 600 Hz (Table I) Subsequent perfusions had less of an effect on the action potential or the cochlear microphonics These potentials tended to decrease in size as the number of perfusions increased

Because the summating potential varied in different preparations, it was not feasible to quantitate it

The endocochlear potential was increased in a positive direction (5 mV) by the first perfusion with artificial perilymph Subsequent perfusions produced less of an increase

The degree of both the inhibition of the action potential and the augmentation of the cochlear microphonics produced by electrical stimulation of the crossed olivocochlear bundle changed in parallel with the change in the magnitude of the crossed olivocochlear potential This was found to occur in all of the experiments to be described in this paper Therefore, the crossed olivocochlear potential was chosen as the parameter to present as an evaluation of the activity of the crossed olivocochlear bundle

The artificial perilymph solution increased the crossed olivocochlear potential approximately 24% during the first perfusion of the scala tympani (Table II) At the end of a second perfusion the average value of the crossed olivocochlear potential fell to 88% of control

### *Action of acetylcholine and choline*

Neither acetylcholine (247 μM, 550 μM) nor choline (550 μM) produced changes in the action potential cochlear microphonics, or endocochlear potential which could be distinguished from the effects of the artificial perilymph

Acetylcholine 247 μM produced a slight inhibition of the crossed olivocochlear po-

Table II *Effect of cholinergic and anticholinergic drugs perfused through the scala tympani on the magnitude of the crossed olivocochlear potential (COCP)*

| 1st perfusing agent | Magnitude of the COCP[a] During 1st perfusion 4–6 min | Post 1st perfusion 0–10 min | Post 2nd perfusion 0–10 min | No of animals |
|---|---|---|---|---|
| Artificial perilymph | 123 (106–134) | 102 | 88 | 4 |
| *d* Tubocurare 5 μM | 24 (20–27) | 0 | 59 | 2 |
| Strychnine 10 μM | 0 (0–0) | 0 | 59 | 2 |
| Decamethonium 25 μM | 55 (53–57) | 0 | 77 | 2 |
| Hexamethonium 550 μM | 68 (64–72) | 43 | 76 | 2 |
| Methylatropine 50 μM | 23 (20–25) | 0 | 84 | 2 |
| Atropine 200 μM | 45 (44–46) | 19 | 72 | 2 |
| Eserine 800 μM | 28 (17–40) | 21 | 81 | 4 |
| Nicotine 1 mM | 68 (60–75) | —[b] | 86[b] | 3 |
| Acetylcholine 546 μM | 54 (50–58) | 21 | 89 | 3 |
| Choline 550 μM | 54 (45–67) | 13 | 80 | 3 |
| Acetylcholine 247 μM and Eserine 20 μM | 0 (0–0) | 0 | 93 | 3 |
| Acetylcholine 54 μM and Eserine 20 μM | 22 (14–29) | 0 | 75 | 2 |
| Carbachol 50 μM | 57 (55–59) | 26 | 87 | 2 |

[a] The magnitude of the COCP is presented as a mean per cent of control The range for the first (4–6 min) value is included
The second perfusion agent was artificial perilymph and was started 10 min after the end of the 1st perfusion
[b] No second perfusion was carried out after the nicotine recovery recorded in 30–50 min

tential (25% in 10 min in two animals) At 546 μM acetylcholine produced a greater inhibition of the crossed olivocochlear potential which was identical to that produced by choline (550 μM) (Table II) Thus, choline and acetylcholine were equipotent on a molar basis

*Action of eserine*

The effects of eserine (20 μM, two experiments) could not be distinguished from the effects of the artificial perilymph With higher concentrations of eserine (800 μM) the pH of the artificial perilymph changed (pH

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## RESULTS

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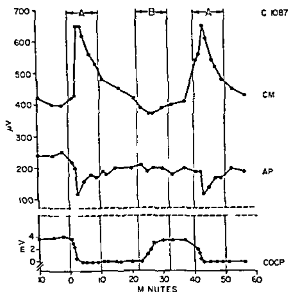



*Fig 1* Effect of perfusing the scala tympani with the acetylcholine (247 μM)-eserine (20 μM) combination (*A*) and artificial perilymph (*B*) *CM* Cochlear microphonics evoked by 600 Hz tone burst (67 dB SPL) recorded without electrical stimulation of the crossed olivocochlear bundle *AP* Action potential evoked by a 6 kHz tone burst (82 dB SPL) recorded without electrical stimulation of the crossed olivocochlear bundle *COCP* Crossed olivocochlear potential evoked by electrical stimulation of the crossed olivocochlear bundle Note that the effect of this electrical stimulation of the crossed olivocochlear bundle on the action potential and cochlear microphonics has been omitted

crossed olivocochlear potential (2 animals) Nicotine (1 mM) showed just a slight inhibitory effect on the crossed olivocochlear potential (Table II) Carbachol (50 μM) produced an inhibition of the crossed olivocochlear potential that was slightly less than that produced by the same molar concentration of acetylcholine (54 μM) when combined with eserine (20 μM) (Table II)

### *Action of several anticholinergics*

None of these agents produced changes in the cochlear microphonics, action potential, or endocochlear potential which could be distinguished from the effects of the artificial perilymph

Strychnine (10 μM) and *d* tubocurare (5 μM) were the most potent (on a molar basis) in inhibiting the crossed olivocochlear potential (Table II) The two bis-onium compounds, hexamethonium and decamethonium, were found to be less effective with decamethonium being 20 times more potent than hexamethonium (Table II) The quaternary atropine, methylatropine, was found to be four times as potent as the tertiary atropine in inhibiting the crossed olivocochlear potential (Table II) The time course of the inhibition and recovery of the crossed olivocochlear potential after the application of inhibitory agents is illustrated by methylatropine in Fig 2

## DISCUSSION

That the events following electrical stimulation of the crossed olivocochlear bundle are mediated by the release of acetylcholine from the bundle s nerve endings has been proposed

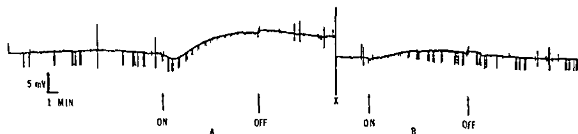



*Fig 2* Changes in the endocochlear potential and in the negative deflections produced by electrical stimulation of the crossed olivocochlear bundle (crossed olivocochlear potentials) produced by perfusion of the scala tympani (during the on–off period) with methylatropine (50 μM *A*) and artificial perilymph (*B*) *X* manual adjustment of the recording pen Deflections with an upward (positive) component represent electrical artifacts Time scale and voltage calibration in lower left corner

by several groups of workers This hypothesis gained credence when it was shown that curare and atropine applied intracochlearly (Konishi, 1972, Fex, 1968) and hemicholinium administered intravascularly (Guth & Amaro, 1969, Brown et al, 1969) blocked the crossed olivocochlear bundle This study has extended the group of anticholinergics which block the crossed olivocochlear bundle to include decamethonium, hexamethonium and methylatropine

Strychnine has been reported to block the crossed olivocochlear bundle (Desmedt & Monaco, 1962, Fex, 1967 *a*) We have confirmed this observation and shown that its potency was about equal to curare That strychnine possesses anticholinergic properties has been demonstrated (Feldberg & Vartiainen, 1934, Lanari & Luco, 1939) Thus, the blocking ability of strychnine confirms the susceptibility of the crossed olivocochlear bundle to anticholinergics

The enzyme, acetylcholinesterase, which may be used for termination of the action of the proposed transmitter substance has been located in association with these efferent fibers (Schuknecht et al, 1959) However, we were unable to demonstrate a potentiation of the crossed olivocochlear bundle by eserine which inhibits cholinesterase The cholinesterase levels in the cochlea decline upon efferent fiber but not hair cell degeneration (Schuknecht et al, 1959) This may be interpreted to mean that the enzyme has a location on the crossed olivocochlear bundle nerve endings and not post synaptic" on the hair cells This localization is similar to that in the autonomic ganglia (Koelle & Koelle, 1959) where it is difficult to demonstrate an eserine potentiation of the neural response (Feldberg & Vartiainen, 1934) This action of eserine has been taken to mean that the termination of the transmitter action is by means of diffusion away from the receptor site rather than by hydrolysis due to the cholinesterase (Emmelin & MacIntosh, 1956) Since cholinesterase inhibitors would not prevent the diffusion process, then an accumulation of transmitter substance and a resultant potentiation of nerve firing would not be observed Therefore, our results with eserine do not negate the hypothesis that acetylcholine is the transmitter substance of the crossed olivocochlear bundle

The curare like action of high doses of eserine found in this study has been documented for other known cholinergic synapses (Feldberg & Vartiainen, 1934, Eccles & MacFarlane, 1949)

If acetylcholine is the transmitter substance exogenously applied acetylcholine should mimic the effects of nerve stimulation We examined the effects of acetylcholine and several agents which possess acetylcholine like activity carbachol, nicotine, choline, arecoline, and decamethonium All of these except arecoline produced an inhibition of the crossed olivocochlear bundle but did not mimic the effects of nerve stimulation at the concentrations used Choline was found to be equipotent to acetylcholine in producing this effect This suggested that all of the acetylcholine applied was hydrolysed by the cholinesterase in the cochlea and arrived at the blocking site as choline This was confirmed by the addition of eserine to acetylcholine and choline solutions It was found that the eserine potentiated the inhibitory effect of acetylcholine more than ten times but not that of choline

Classically, acetylcholine and acetylcholine like agents demonstrate blocking activity at known cholinergic sites (Feldberg & Vartiainen, 1934, Thesleff, 1955, Paton & Savini 1968, Nastuk & Gissen, 1966, Paton & Zaimis 1949, Barlow, 1964 ) The rate of drug delivery (2 $\mu$l/min) used in this study was so slow that apparently no acetylcholine reached the blocking site before hydrolysis by cholinesterase We demonstrated a mimicking response only by combining a high concentration of acetylcholine with eserine The addition of eserine insured a delivery of acetylcholine molecules The large concentration of acetylcholine increased the rate of delivery

without an increase in the perfusion rate These results are in agreement with the classical demonstrations that the mimicking effects of acetylcholine are highly dependent on concentration and a fast rate of delivery to cholinergic sites (Brown et al, 1936, Riker, 1966) Similarly, concentrations higher than those used in this study will probably have to be used to demonstrate a mimicking activity with the acetylcholine like agents We may have also explained why others failed to demonstrate a mimicking activity with acetylcholine applied into the cochlea (Sohmer & Feinmesser, 1963)

Parallel with the mimicking response to acetylcholine, a blockade of the crossed olivocochlear bundle occurred This action of acetylcholine may be similar to that classically labeled a "polarization blockade" (Paton & Perry, 1953, Barlow, 1964) Electrical stimulation of the crossed olivocochlear bundle is thought to result in a hyperpolarization of the hair cells which causes an augmentation of the cochlear microphonics and an inhibition of the action potential (Fex, 1967) A hyperpolarization of the hair cells by exogenously applied acetylcholine would produce a blockade of the crossed olivocochlear bundle by preventing additional hyperpolarization due to the nerve released transmitter

The hyperpolarization of the hair cells is reflected by a negative change in the endocochlear potential called the crossed olivocochlear potential (Fex, 1967) Our inability to detect a negative change in the endocochlear potential recording does not negate the "polarization" hypothesis Thus, the artificial perilymph may have increased the endocochlear potential through a mechanism independent of the hair cell potential This increase in the endocochlear potential by the artificial perilymph may have masked any changes in the hair cell potential which would have otherwise been reflected and measured in the endocochlear potential recording However, the augmentation of the cochlear microphonics and inhibition of the action potential evoked by the acetylcholine is evidence for a "polarization" blockade of the crossed olivocochlear bundle

The mimicking response evoked by acetylcholine faded rapidly with the continued application of the drug Also the cochlear tissues did not respond to further applications of acetylcholine Thus, it appears that the continued presence of acetylcholine, which was insured by the inhibition of cholinesterase by eserine, resulted in an "inhibition" of the sites initially activated by the acetylcholine This type of acetylcholine response has been documented for other cholinergic systems and labeled "desensitization" (Barlow, 1964, Thesleff, 1955)

The site of action of all of the agents used in this study is unknown The order of potency in inhibiting the crossed olivocochlear potential was the reverse of that found on unmyelinated nerve fibers where nicotine was found to be the most potent agent (Armett & Ritchie, 1961) Also the action potential was not reduced except by the acetylcholine (247 $\mu$M)-eserine (20 $\mu$M) combination If the agents were acting in a non specific (procaine like) manner to block conduction along the unmyelinated portions of the nerve fibers in the cochlea, both the action potential and the crossed olivocochlear potential would be blocked Thus, it appears that the site of action of the majority of the drugs was not on the nerve fibers

The effects of both the exogenously applied acetylcholine and the crossed olivocochlear bundle were blocked by curare This suggests that the inhibitory transmitter substance and the exogenously applied acetylcholine were acting at the same curare sensitive sites

In conclusion we have shown that the action of the drugs studied are compatible with the hypothesis that acetylcholine is the transmitter substance of the crossed olivocochlear bundle Several criteria must be satisfied before a chemical is regarded as a transmitter substance (McLennan, 1963) In this study we have demonstrated the mimicking of nerve

by several groups of workers This hypothesis gained credence when it was shown that curare and atropine applied intracochlearly (Konishi, 1972, Fex, 1968) and hemicholinium administered intravascularly (Guth & Amaro, 1969, Brown et al, 1969) blocked the crossed olivo cochlear bundle This study has extended the group of anticholinergics which block the crossed olivocochlear bundle to include decamethonium, hexamethonium and methylatropine

Strychnine has been reported to block the crossed olivocochlear bundle (Desmedt & Monaco, 1962, Fex, 1967 *a*) We have confirmed this observation and shown that its potency was about equal to curare That strychnine possesses anticholinergic properties has been demonstrated (Feldberg & Vartiainen, 1934, Lanari & Luco, 1939) Thus, the blocking ability of strychnine confirms the susceptibil ity of the crossed olivocochlear bundle to anticholinergics

The enzyme, acetylcholinesterase, which may be used for termination of the action of the proposed transmitter substance has been located in association with these efferent fibers (Schuknecht et al, 1959) However, we were unable to demonstrate a potentiation of the crossed olivocochlear bundle by eserine which inhibits cholinesterase The cholinesterase levels in the cochlea decline upon efferent fiber but not hair cell degeneration (Schuknecht et al, 1959) This may be in terpreted to mean that the enzyme has a location on the crossed olivocochlear bundle nerve endings and not 'post synaptic" on the hair cells This localization is similar to that in the autonomic ganglia (Koelle & Koelle, 1959) where it is difficult to demonstrate an eserine potentiation of the neural response (Feldberg & Vartiainen, 1934) This action of eserine has been taken to mean that the ter mination of the transmitter action is by means of diffusion away from the receptor site rather than by hydrolysis due to the cholinesterase (Emmelin & MacIntosh 1956) Since cholin esterase inhibitors would not prevent the dif fusion process, then an accumulation of trans mitter substance and a resultant potentiation of nerve firing would not be observed There fore, our results with eserine do not negate the hypothesis that acetylcholine is the trans mitter substance of the crossed olivocochlear bundle

The curare-like action of high doses of eserine found in this study has been docu mented for other known cholinergic synapses (Feldberg & Vartiainen, 1934, Eccles & Mac Farlane, 1949)

If acetylcholine is the transmitter substance, exogenously applied acetylcholine should mim ic the effects of nerve stimulation We ex amined the effects of acetylcholine and sev eral agents which possess acetylcholine like activity carbachol, nicotine, choline, arecoline, and decamethonium All of these except arecoline produced an inhibition of the crossed olivocochlear bundle but did not mimic the effects of nerve stimulation at the concen trations used Choline was found to be equipotent to acetylcholine in producing this effect This suggested that all of the acetylcholine applied was hydrolysed by the cholinesterase in the cochlea and arrived at the blocking site as choline This was confirmed by the addi tion of eserine to acetylcholine and choline solutions It was found that the eserine potentiated the inhibitory effect of acetylcholine more than ten times but not that of choline

Classically, acetylcholine and acetylcholine like agents demonstrate blocking activity at known cholinergic sites (Feldberg & Vartiai nen, 1934, Thesleff, 1955, Paton & Savini 1968, Nastuk & Gissen, 1966, Paton & Zaimis 1949, Barlow, 1964) The rate of drug de livery (2 $\mu$l/min) used in this study was so slow that apparently no acetylcholine reached the blocking site before hydrolysis by cholin esterase We demonstrated a mimicking re sponse only by combining a high concentra tion of acetylcholine with eserine The addi tion of eserine insured a delivery of acetyl choline molecules The large concentration of acetylcholine increased the rate of delivery

without an increase in the perfusion rate. These results are in agreement with the classical demonstrations that the mimicking effects of acetylcholine are highly dependent on concentration and a fast rate of delivery to cholinergic sites (Brown et al., 1936, Riker, 1966). Similarly, concentrations higher than those used in this study will probably have to be used to demonstrate a mimicking activity with the acetylcholine-like agents. We may have also explained why others failed to demonstrate a mimicking activity with acetylcholine applied into the cochlea (Sohmer & Feinmesser, 1963).

Parallel with the mimicking response to acetylcholine, a blockade of the crossed olivocochlear bundle occurred. This action of acetylcholine may be similar to that classically labeled a "polarization blockade" (Paton & Perry, 1953, Barlow, 1964). Electrical stimulation of the crossed olivocochlear bundle is thought to result in a hyperpolarization of the hair cells which causes an augmentation of the cochlear microphonics and an inhibition of the action potential (Fex, 1967). A hyperpolarization of the hair cells by exogenously applied acetylcholine would produce a blockade of the crossed olivocochlear bundle by preventing additional hyperpolarization due to the nerve released transmitter.

The hyperpolarization of the hair cells is reflected by a negative change in the endocochlear potential called the crossed olivocochlear potential (Fex, 1967). Our inability to detect a negative change in the endocochlear potential recording does not negate the "polarization" hypothesis. Thus, the artificial perilymph may have increased the endocochlear potential through a mechanism independent of the hair cell potential. This increase in the endocochlear potential by the artificial perilymph may have masked any changes in the hair cell potential which would have otherwise been reflected and measured in the endocochlear potential recording. However, the augmentation of the cochlear microphonics and inhibition of the action potential evoked by the acetylcholine is evidence for a "polarization" blockade of the crossed olivocochlear bundle.

The mimicking response evoked by acetylcholine faded rapidly with the continued application of the drug. Also the cochlear tissues did not respond to further applications of acetylcholine. Thus, it appears that the continued presence of acetylcholine, which was insured by the inhibition of cholinesterase by eserine, resulted in an "inhibition" of the sites initially activated by the acetylcholine. This type of acetylcholine response has been documented for other cholinergic systems and labeled "desensitization" (Barlow, 1964, Thesleff, 1955).

The site of action of all of the agents used in this study is unknown. The order of potency in inhibiting the crossed olivocochlear potential was the reverse of that found on unmyelinated nerve fibers where nicotine was found to be the most potent agent (Armett & Ritchie, 1961). Also the action potential was not reduced except by the acetylcholine (247 μM)-eserine (20 μM) combination. If the agents were acting in a non specific (procaine like) manner to block conduction along the unmyelinated portions of the nerve fibers in the cochlea, both the action potential and the crossed olivocochlear potential would be blocked. Thus, it appears that the site of action of the majority of the drugs was not on the nerve fibers.

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In conclusion we have shown that the action of the drugs studied are compatible with the hypothesis that acetylcholine is the transmitter substance of the crossed olivocochlear bundle. Several criteria must be satisfied before a chemical is regarded as a transmitter substance (McLennan, 1963). In this study we have demonstrated the mimicking of nerve

stimulation by exogenously applied acetylcholine, and the blockade of exogenously applied acetylcholine by the same agents which block nerve stimulation Others have shown the presence of cholinesterase (Schuknecht et al, 1959) the release of acetylcholine upon crossed olivocochlear bundle stimulation (Norris 1972) and the presence of cholineacetylase which synthesizes acetylcholine (Guth & Jasser, 1973)

## ACKNOWLEDGMENT

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## ZUSAMMENFASSUNG

Wie gezeigt werden konnte bewirkt ein Introduzieren von sowohl cholinergischen wie anticholinergischen Drogen in die Scala tympani von Meerschweinchen eine Blockade der Effekte die bei elektrischer Reizung des gekreuzten olivocochlearen Bundels ausgelost werden Strychnin d Tubocurare und Dekamethonium wirkten in dieser Hinsicht am starksten Eserin verstarkte nicht die durch elektrische Reizung des gekreuzten Olivocochlearbundels ausgelosten Effekte potenzierte jedoch die Einwirkung von Acetylcholin und blockierte bei hoher Konzentration das gekreuzte Olivocochlearbundel Durch Acetylcholin (247 μM) in Kombination mit Eserin (20 μm) wurden die Effekte des gekreuzten Olivocochlearbundels gleichsam imitiert d h es ergab sich eine Erhohung der Cochlearmikrophonie und eine Hemmung (Inhibition) des Aktionspotentials Die Acetylcholin (247 μm) — Eserin (20 μM) — Kombination loste auch eine reversible Blockade des gekreuzten Olivocochlearbundels und der Imitations Effekte bei subsekutivem Spritzen mit dieser Drogenkombination aus Der Imitations Effekt der obigen Acetylcholin Eserin Kombination wurde durch d Tubocurare blockiert Diese Ergebnisse sind eine weitere Bestatigung dafur dass Acetylcholin die Transmittersubstanz des gekreuzten Olivocochlearbundels ist

## REFERENCES

Amaro J Guth P S & Wanderlinder L 1966 Inhibition of auditory nerve action potentials by acetylcholine and physostigmine *Brit J Pharmacol 28* 207

Armett C J & Ritchie J M 1961 The action of acetylcholine and some related substances on conduction in mammalian non myelinated nerve fibers *J Physiol* (Lond) *155* 372

Barlow R B 1964 *Introduction to chemical pharmacology* 2nd edition p 452 Methuen & Co London

Bobbin R P & Konishi T 1971 Acetylcholine mimics crossed olivocochlear bundle stimulation *Nature New Biol 231* 222

Brown R D Daigneault E A & Pruett J R 1969 The effects of selected cholinergic drugs and strychnine on cochlear responses and olivo-cochlear inhibition *J Pharmacol Exp Ther 165* 300

Brown G L Dale H H & Feldberg W 1936 Reactions of the normal mammalian muscle to acetylcholine and to eserine *J Physiol* (Lond) *87* 394

Daigneault E A & Brown R D 1966 Acetylcholine suppression of the $N_1$ component of round window recorded cochlear potentials *Arch Int Pharmacodyn Ther 162* 20

Desmedt J E & Monaco P 1962 The pharmacology of a centrifugal inhibitory pathway in the cat s acoustic system *Proc 1st Int Pharmacol Congr 8* 183

Eccles J C & MacFarlane W V 1949 Actions of anticholinesterases on endplate potential of frog muscle *J Neurophysiol 12* 59

Emmelin N & MacIntosh F C 1956 The release of acetylcholine from perfused sympathetic ganglia and skeletal muscles *J Physiol* (Lond) *131* 477

Feldberg W & Vartiainen A 1934 Further observations on the physiology and pharmacology of a sympathetic ganglion *J Physiol* (Lond) *83* 103

Fex J 1959 Augmentation of the cochlear microphonics by stimulation of efferent fibers to cochlea *Acta Otolaryng* (Stockh) *50* 540

— 1967 Efferent inhibition in the cochlea related to hair-cell dc activity Study of postsynaptic activity of the crossed olivocochlear fibers in the cat *J Acoust Soc Amer 41* 666

— 1967 a Calcium action at an inhibitory synapse *Nature* (Lond) *213* 1233

— 1968 Efferent inhibition in the cochlea by the olivocochlear bundle In *Hearing mechanisms in vertebrates* (ed A V S DeReuck & J Knight) pp 169 181 Little Brown & Co Boston

Galambos R 1956 Suppression of the auditory nerve activity by stimulation of efferent fibers to the cochlea *J Neurophysiol 19* 424

Gisselsson L 1960 Effect on microphonics of acetylcholine injected into the endolymphatic space *Acta Otolaryng* (Stockh) *51* 636

Guth P S & Amaro J 1969 A possible cholinergic link in olivocochlear inhibition *Neuropharmacology 8* 49

Guth P S & Bobbin R P 1971 The pharmacology of peripheral auditory processes cochlear pharmacology *Adv Pharmacol Chemother 9* 93

Guth P S & Jasser A 1973 Synthesis of acetylcholine by the olivocochlear bundle *J Neurochem* in press

Koelle W A & Koelle G B 1959 The localization of external or functional acetylcholinesterase at the synapses of autonomic ganglia *J Pharmacol Exp Ther 126* 1

mit dem Modiolusteil des Nervus

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Acta Otolaryng 77 66–71, 1974

# DAS PERIPHERE OLIVOCOCHLEARE BÜNDEL DER LABORATORIUMSRATTE

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(Eingegangen am 29 Juni 1973)

*Abstrakt* Mit Hilfe der Darstellung der Acetylcholin esterase wurden die efferenten Fasern in der Cochlea der Laboratoriumsratte untersucht Das Verteilungs muster ist prinzipiell wie bei anderen Saugetieren Unterschiede ergaben sich bei den intralaminaren Spiralbundeln und bei den direkten efferenten Fasern im Nervus cochlearis Beide Faserarten waren mit dem Acetylcholinesterase Nachweis nicht darstellbar

Das olivocochleare Bundel (OCB) wurde zwar schon durch Rasmussen (1942) entdeckt, aber erst durch die Verwendung des Nachweises der Acetylcholinesterase (AChE) wurde eine einfache Moglichkeit eroffnet, die efferenten Fasern darzustellen (Schuknecht et al, 1959) Die zahlreichen seither mit dieser Methode erhobenen Befunde sind von Iurato und Mitarbeitern (1971) zusammengestellt worden Bei der Ratte wurden zwar in jungster Zeit von Brown & Howlett (1972) die zentralen Abschnitte des OCB untersucht, uber den peripheren Teil liegen jedoch bisher nur elektronenmikroskopische Ergebnisse (Iurato, 1962) vor Die vorliegende Untersuchung ist in Hinblick auf die geplante operative Durchtrennung des OCB bei der Ratte entstanden Die funktionelle Bedeutung des efferenten Systems ist bis heute noch nicht hinreichend geklärt (Klinke et al, 1971) eine Funktion unter physiologischen Bedingungen wird von manchen Autoren uberhaupt bestritten (Pfalz, 1969, Igarashi et al, 1972) Eine genaue morphologische Untersuchung des efferenten Systems erscheint bei einem wichtigen Versuchstier, wie der Laboratoriumsratte, angebracht

## MATERIAL UND METHODE

Albinoratten von einem Gewicht zwischen 250 und 400 g wurden mit Äther und Chloralhydrat anasthesiert Danach perfundierten wir vom Herzen aus mit 5%iger Formollosung (Phosphatpuffer pH 7,4) Die isolierten Schlafenbeine wurden zwei Tage nachfixiert und dann in EDTA entkalkt Von den weitgehend isolierten Cochleae fertigten wir 10 $\mu$ dicke Gefrierschnitte an Diese wurden nach 12stundiger Aufbewahrung im Kuhlschrank nach Karnovsky und Roots sehr lange (24 Stunden) inkubiert Als Substrat fur die AChE diente Acetylthiocholinjodid Versuchsweise wurden einige Schnitte mit Propionylthiocholinjodid inkubiert, da nach Gruber et al (1971) und nach Eranko (1973) die AChE bei der Ratte auch dieses Substrat spaltet Zur Sicherung der Spezifität der Reaktion fugten wir dem Inkubationsmedium $10^{-4}$ m iso Ompa zu um die unspezifische Cholinesterase zu hemmen Die meisten Schnitte wurden mit Hämatoxylin schwach gegengefarbt

## ERGEBNIS

Die AChE laßt sich in den efferenten Fasern des Innenohres und in den Perikarya der

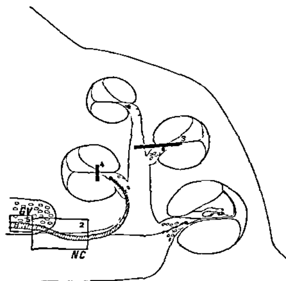



*Abb 1* Schema der Cochlea schraffiert dargestellt ist das olivocochleare Bundel *GV*, Ganglion vestibulare *NC* Nervus cochlearis Die Zahlen 2 3 4 und 5 verweisen auf die folgenden Abbildungen

Ganglienzellen des Ganglion vestibulare und des Ganglion spirale nachweisen Bei der langen Inkubationszeit, die wir benutzten, zeigen die efferenten Fasern eine starke und konstante Reaktion In den Perikarya der Ganglienzellen variiert die Reaktionsstarke An den Spiralganglienzellen konnten wir diese Erscheinung beschreiben (Seiller et al , 1972, Firbas et al , 1973) Von anderen sensiblen Ganglienzellen ist der Gehalt an AChE schon fruher beschrieben worden (Giacobini, 1959, Liedel, 1971, Gwyn & Flumerfelt, 1971)

Da die efferenten Fasern bis zu ihren Endigungen gut darstellbar sind, laßt sich der Verlauf genau verfolgen Die efferenten Fasern liegen zuerst im Nervus vestibularis Direkte, primar im Nervus cochlearis verlaufende Fasern fanden wir bei der Ratte nicht Vom Nervus vestibularis ziehen die efferenten Fasern in der Oort'schen Anastomose an den Nervus cochlearis (Abb 1 und 2) Der großere Teil der Fasern biegt nach apical ab und gelangt, den Cochlearisstamm verlassend, sofort zum Spiralganglion der oberen Basalwindung Diese Fasern haben in der Fortsetzung keinen Kontakt mit dem Modiolusteil des Nervus cochlearis

Der Rest der Fasern, der zum Spiralganglion der unteren Basalwindung zieht, durchsetzt facherformig in mehreren Bundeln den Nervus cochlearis beim Eintritt in den Modiolus (Abb 1 und 2) Vom intraganglionaren Spiralbundel laufen radiale Fasern zwischen den beiden Knochenlamellen der Lamina spiralis ossea zum Cortischen Organ (Abb 3) Ausgesprochen spiralige Fasern in der Lamina spiralis, wie sie Nomura & Schuknecht (1965) beim Menschen fanden, konnten wir nicht nachweisen An Radialschnitten ist zu erkennen, daß die AChE positiven Fasern von der tympanalen Lamelle zur vestibularen Lamelle aufsteigen und durch die Habenula perforata zum Cortischen Organ gelangen An Querschnitten durch die Lamina spiralis sind die AChE-positiven Fasern zu erkennen (Abb 4)

Im Bereich des Cortischen Organs sind efferente Fasern in den spiraligen und radiaren Tunnelbundeln nachweisbar Die efferenten Nervendigungen an den außeren Haarzellen wurden von uns nicht im einzelnen registriert Es steht aber auch hier das bekannte Muster fest Im apicalen Bereich der Cochlea und jeweils an der dritten Reihe der außeren Haarzellen sind geringere Depots von Reaktionsprodukten abgelagert

Bei starkerer Vergroßerung ließ sich fallweise erkennen, daß die Reaktionsprodukte in Doppelkonturen entlang von Nervenfasern vorliegen (Abb 5) Das steht in Einklang mit den elektronenmikroskopischen Befunden von Nakai (1972) Dieser fand AChE-Aktivitat bei markhaltigen Fasern an der Axoplasmamembran An den Bildern von Nakai kann man feststellen, daß die AChE bei den markhaltigen Fasern nicht in die Umgebung der Fasern diffundiert Obwohl wir mit langer Inkubationszeit arbeiteten, scheint durch die Fixation der Präparate die Diffusion von AChE auch bei den unmyelinisierten Fasern weitgehend vermieden worden zu sein Der Gehalt an AChE in den Spiralganglienzellen ist offensichtlich geringer als in den efferenten Fasern

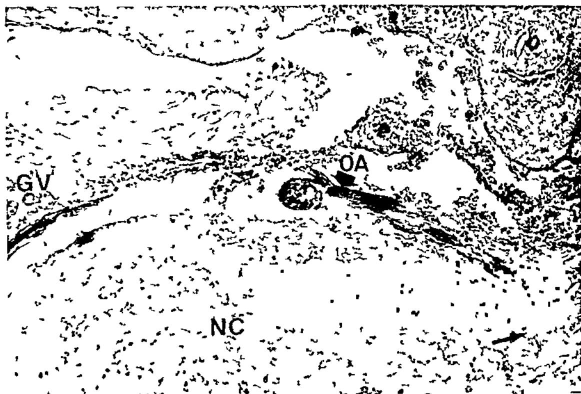



*Abb 2* Olivocochleares Bundel im Übergang vom Nervus vestibularis zum Nervus cochlearis *GV* Ganglion vestibulare *NC* Nervus cochlearis *OA* Oortsche Anastomose Der Pfeil verweist auf ein efferentes Bundel zur unteren Basalwindung

Die efferenten Fasern zeigen schon nach 2 stundiger Inkubation deutliche Reaktionen wahrend die Spiralganglienzellen noch ungefarbt sind Im Gegensatz zu Brown & Howlett (1972) konnten wir bei gleichen Versuchsbedingungen immer vergleichbare Resultate erzielen

## DISKUSSION

In den wesentlichen Merkmalen ist der Verlauf der peripheren AChE positiven (efferenten) Fasern bei der Ratte ahnlich wie bei den anderen bisher untersuchten Saugetieren Katze (Rasmussen 1946 1953 Gacek et al 1965) Kaninchen (Rossi & Cortesina 1965) Mensch (Gacek 1961 Nomura & Schuknecht 1965 Ishii et al 1967) Meerschweinchen (Terayama et al 1969 Terayama & Yamamoto 1971) und Totenkopfaffchen (Ishii et al 1967) Auffallende *Unterschiede sind nach unseren* Befunden keine AChE positiven Fasern die mit dem Stamm des Nervus cochlearis herangefuhrt werden Nach Ishii et al (1967) gehoren solche Fasern nicht zum olivocochlearen Bundel Auffallend war bei der Ratte auch das Fehlen spiralig verlaufender AChE positiver Fasern in der Lamina spiralis ossea Nomura & Schuknecht (1965) konnten spiralige Bundel in großer Zahl bei Katze und Mensch darstellen Es scheinen also beachtliche Artunterschiede im Verteilungsmuster des OCB zu bestehen Auch der Ursprung des OCB unterscheidet sich bei der Ratte von dem der Katze und des Meerschweinchens (Brown & Howlett 1972) es entspringt als einheitliches Bundel in den Kernen des Trapezkorpers

Die Eigenschaften der in der Lamina spiralis ossea verlaufenden Spiralbundel (intralaminare Spiralbundel) werden von Johnsson & Hawkins (1972) ausfuhrlich erortert Diese Autoren *halten die intralaminaren Spiralfasern fu*

IGSR

GS

CO

NC

*Abb. 3.* Tangentialschnitt durch die Cochlea. *NC* Nervus cochlearis. *GS* Ganglion spirale. *IGSB* intraganglionäres Spiralbündel. *CO* Cortisches Organ. Der Pfeil verweist auf radiäre, intralaminäre Fasern.



*Abb. 4.* Querschnitt durch die Lamina spiralis ossea. Der Pfeil verweist auf ein efferentes radiär verlaufendes Nervenbündel.

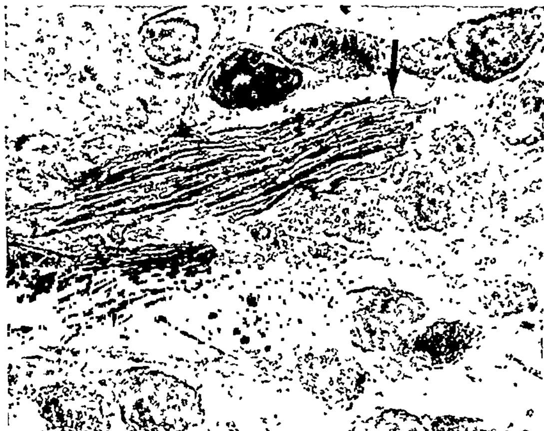


*Abb 5* Ganglion vestibulare mit olivocochlearem Bundel Mit dem Pfeil ist eine Doppelkontur entlang einer Nervenfaser anеezeichnet

afferent Ebenso umstritten ist die Bedeutung markhaltiger und markloser Fasern in diesem Bereich (Terayama & Yamamoto, 1971) Wie eingangs erwahnt ist die Diskussion um die Funktion der efferenten Fasern im Cortischen Organ nicht zu Ende Neue pharmakologische Befunde lassen es als sicher erscheinen, daß zumindest ein Erregungsstoff der Fasern Acetylcholin ist (Galley et al, 1971, Bobbin & Konishi, 1971, Konishi, 1972, Klinke et al, 1973) Der Nachweis des Ferments Acetyl cholinesterase steht also in sinnvollem Zusammenhang mit dieser Transmittersubstanz

## SUMMARY

The efferent olivocochlear bundle in the rat was demonstrated by histochemical localization of acetyl cholinesterase The distribution of the peripheral olivocochlear bundle is similar to that of other mammals Differences exist in the direct efferent cochle fibres and in the intralaminar spiral fibres Neit of these fibre types could be demonstrated in the with help of acetylcholinesterase activity

## LITERATUR

Bobbin R P & Konishi T 1971 Acetylcholi mimics crossed olivocochlear bundle stimulatic *Nature New Biol* (Lond) *231*, 222

Brown J C & Howlett B 1972 The olivo-cochle tract in the rat and its bearing on the homologi of some constituent cell groups of the mammali superior olivary complex A thiocholine stud *Acta Anat 83* 505

Eranko L 1973 Effect of pH on the activity nervous cholinesterases of the rat towards di fferent biochemical and histochemical substrat and inhibitors *Histochemie 33* 1

Firbas W Rameis H & Sinzinger, H 1973 Üb die Acetylcholinesterase Aktivitat in den Spira ganglienzellen der Ratte *Excerpt Med 276* 33 Tenth World Congr Otorhinolaryng (Venice)

Gacek R R 1961 The efferent cochlear bundle in man *Arch Otolaryng* (Chic ) *74* 690
Gacek, R R Nomura Y & Balogh K 1965 Acetylcholinesterase activity in the efferent fibers of the stato acoustic nerve *Acta Otolaryng* (Stockh ) *59* 541
Galley N Klinke R Pause M & Storch W H 1971 The effect of Flaxedil (Gallamine triethiodide) on the efferent endings in the cochlea *Pflugers Arch Ges Physiol 330* 1
Giacobini E 1959 The distribution and localization of cholinesterases in nerve cells *Acta Physiol Scand 45* Suppl *156* 1
Gruber H Zenker W & Hohberg E 1971 Untersuchung uber die Spezifitat der Cholinesterasen im peripheren Nervensystem der Ratte *Histochemie 27* 78
Gwyn D G & Flumerfelt B A 1971 Acetylcholinesterase in non cholinergic neurones A histochemical study of dorsal root ganglion cells in the rat *Brain Res* (Amsterd ) *34* 193
Igarashi M Alford B R Nakai Y & Gordon W P 1972 Behavioral aud tory function after transection of crossed olivo-cochlear bundle in the cat *Acta Otolaryng* (Stockh ) *73* 455
Ishii T Murakami Y & Balogh K 1967 Acetylcholinesterase activity in the efferent nerve fibers of the human inner ear *Ann Otol 76* 69
Ishii T Murakami Y & Gacek R R 1967 Histochemical study of the acetylcholinesterase activity in the inner ear of the squirrel monkey *Acta Otolaryng* (Stockh ) *64* 267
Iurato S 1962 Efferent fibers to the sensory cells of Corti s organ *Exp Cell Res 27* 16?
Iurato S Luciano L Pannese E & Reale E 1971 Histochemical localization of acetylcholinesterase (AChE) activity in the inner ear *Acta Otolaryng* (Stockh ) Suppl *279*
Johnsson L G & Hawkins J E 1972 Sensory and neural degeneration with aging as seen in micro dissections of the human inner ear *Ann Otol 81* 179
Klinke R Galley N Pause M & Storch W H 1973 Tetanus toxin blocks the efferent endings in the cochlea *Bra n Res* (Amsterd ) *49* 447
Klinke R Galley N & Storch W H 1971 Die efferente Innervat on des auditorischen Systems *Proc First Europ Biophys Congr 1971* (Baden Austria) 73
Konishi T 1972 Action of tubocurarine and atropine on the crossed olivocochlear bundles *Acta Otolaryng* (Stockh ) *74* 252
Liedel K 1971 Zum histotopochemischen Vorkommen von Enzymen in sensiblen Ganglien dargestellt am Ggl semilunare des Hausrindes II Hydrolasen *Gegenbaurs Morph Jahrb* (Leipzig) *117* 256
Nakai Y 1972 Fine structural localization of acetylcholinesterase in the adult and developing cochlea *Laryngoscope 82* 177
Nomura Y & Schuknecht H F 1965 The efferent fibers in the cochlea *Ann Otol 74* 289
Pfalz R K J 1969 Absence of a function for the crossed olivocochlear bundle under physiolog cal conditions *Arch Klin Exp Ohr Nas Kehlkopfheilk 193* 89
Rasmussen G L 1942 An efferent cochlear bundle *Anat Rec 82* 441
— 1946 The olivary peduncle and other fiber projections of the superior olivary complex *J Comp Neurol 84* 141
— 1953 Further observations of the efferent cochlear bundle *J Comp Neurol 99* 61
Rossi G & Cortesina G 1965 The efferent cochlear and vestibular system in *Lepus cuniculus* L *Acta Anat 60* 362
Schuknecht, H F Churchill J A & Doran R 1959 The localization of acetylcholinesterase in the cochlea *Arch Otolaryng* (Chic ) *69* 549
Seiller W Wicke W & Firbas W 1972 Zur Änderung der Acetylcholinesterase Aktivitat im Ganglion spirale nach akustischer Belastung Joint meeting of international societies for hygiene preventive and social medicine 1972 (Vienna) 1
Terayama Y & Yamamoto K 1971 Olivo-cochlear bundle in the guinea pig cochlea after central transsection of the crossed bundle *Acta Otolaryng* (Stockh ) *72* 385
Terayama Y Yamamoto K & Sakamoto T 1969 The efferent olivo-cochlear bundle in the guinea pig cochlea *Ann Otol 78* 1254

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# THE SIGNIFICANCE OF PERIPHERAL VISION IN THE PERCEPTION OF MOVEMENT

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*Abstract* Certain significant differences have been shown to occur in the character of the optokinetic response of a subject gazing actively and passively at a moving striped drum which suggest that different nervous mechanisms subserve each Image motion across the retina appears to be the stimulus inducing the passive variety and is moreover largely responsible for the illusory sensations of self rotation This same mechanism may be invoked by way of explanation of the phenomenon of reversed optokinetic nystagmus The occurrence of this phenomenon in patients with non congenital as well as congenital nystagmus lends support to this explanation Eye movements induced in the normal eye of subjects with unilateral ophthalmoplegia following stimulation of the paretic eye provide additional support for the contention that peripheral vision contributes to the control of normal ocular movements

In the past investigations in man aimed at the elucidation of the physiological mechanisms subserving the control of normal ocular movements have for the most part involved studies of one kind or another concerned with optic fixation upon a single luminous moving target perceived either in total darkness or against a homogenous background The conclusions derived from such studies may be questioned on the grounds that the experimental situation is an unusual one in that normally a moving target is viewed against a stationary background Consequently as the eyes follow the target the images of the background will traverse the retinae in the opposite direction in a manner which will tend to oppose movement It follows that two separate and distinct channels of information must be transmitted to the brain one derived from the fovea and the other from the peripheral retina

How this information is integrated at a cortical level is obscure but it would be surprising if the peripheral component were not used to advantage as a controlling mechanism aiding stability of eye movements In what follows a series of investigations will be described in which it has been shown that peripheral vision can exert a significant and important influence upon ocular movements These investigations have for the most part been concerned with observations of optokinetic nystagmus For this purpose a striped revolving drum of the kind shown in Fig 1 was used The drum is made of black material lined at intervals of 20 degrees with white vertical stripes Provision is made for good illumination of the interior of the drum by means of a light placed above the subject's head The drum itself is servo mechanically controlled and can be rotated at angular velocities up to 120°/sec [1] The resultant eye movements were recorded electro nystagmographically and displayed upon a conventional pen recorder using d c amplification

Typical recordings of optokinetic nystagmus are shown in Fig 2 In the upper tracing the



*Fig 1* Optokinetic drum The drum is servo-mechanically controlled and may be rotated at velocities up to 120°/sec [1]

subject was instructed to deliberately follow the stripes of the drum as they came into view eliciting what might be termed an "active form of optokinetic nystagmus In the lower tracing the subject gazed without conscious effort at the moving stripes producing a passive" form of optokinetic nystagmus Although on cursory examination the wave form of the nystagmus under these two conditions may seem to be identical consisting of a slow component in the direction of rotation of the drum and a fast component in its opposite, certain significant points of difference are apparent Thus in both tracings the arrows shown provide an indication of points at which drum direction was abruptly reversed With "active' optokinetic nystagmus this results in a deviation of the eyes in the direction of rotation of the drum with the fast component returning the eyes towards the midline In the case of "passive" optokinetic nystagmus on the other hand, the eyes are deviated in the direction of the fast component of the nystagmus and it is the slow component which returns the eyes towards the midline

These findings have a significant bearing upon the controversy which has existed for many years upon the mechanism of optokinetic nystagmus Thus Rademaker & Ter Braak *(1948) take the view that the slow* phase is a fixation movement, whereas the rapid phase is a movement whereby the slow phase is rhythmically interrupted owing to what they term the rhythmical activity of the cerebral mechanism It was obviously the tacit acceptance of this mode of action that led Kestenbaum (1948) to write of train, synonymous with optokinetic nystagmus, in the following terms "The genesis of this nystagmus is obvious The eyes are kept fixed on an object in the scenery and therefore deviate backwards in slow motion, they then leave the object and quickly return to their previous position" In other words the form of optokinetic response envisaged by these authors would seem to accord with the upper tracing in Fig 2 of "active" optokinetic nystagmus By contrast, Brucher (1964) and others in separate studies have commented upon the deviation of the eyes occurring in the direction of the fast component

On this account Brucher was led to describe the fast component as the "prime movens" of the nystagmus Borries (1926) similarly likens the fast component to a mechanism akin to the fixation reflex which he refers to as haptation whereby images of objects attracting our attention at the periphery are reflexely transferred to the macula

This view assigns to the fast component a much higher cerebral function than that envisaged by Rademaker and Ter Braak involving in all probability the frontal centres for voluntary gaze (Dix & Hood, 1971) Whether or not these observations may be applied to the fast component of "active" optokinetic nystagmus as defined here remains to be de-







*Fig 2* Nystagmus tracings obtained with a subject following the stripes of the drum (Active optokinetic nystagmus) and gazing passively at the drum (Passive optokinetic nystagmus) Arrows indicate reversal of drum direction Note deviation of eyes in the direction of the slow component of the nystagmus in the case of active optokinetic nystagmus and in the direction of the fast component in the case of passive optokinetic nystagmus

termined but in any event it is the "passive" form of optokinetic nystagmus which best fits the description of both Brucher and Borries

There exist however other essential points of difference between "active" and "passive" optokinetic nystagmus In the "active" form the images of the stripes are fixed upon the fovea and consequently the eye velocity matches that of the drum velocity at all times In fact, despite various claims to the contrary (Westheimer, 1954), it is possible in this way to elicit smooth tracking movements of the eyes to velocities matching drum velocities as high as 100°/sec$^{-1}$

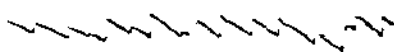
This however calls for considerable conscious effort and is difficult to maintain for any period of time even with quite low drum velocities Instead, examination of recordings carried out under these conditions show that the eye movements fall into two types, those in which the eye velocity accurately matches that of the drum velocity and those in which the eye velocity is consistently inferior to that of the drum In fact what appears to be happening is that despite conscious effort the nystagmic response repeatedly lapses from the "active" to the "passive' type response In the clinical situation therefore even though the patient may be instructed to follow the stripes of the drum it is in general the "passive" type which will be elicited by virtue of his inability to sustain the "active" response

Now it is clearly apparent that the mechanism of this response must be quite different from that of "active" optokinetic nystagmus Since the velocity of the eyes does not reach that of the drum there exists a velocity error between the two so that the images of the stripes continually traverse the retina In consequence since the fovea is not dominantly involved as in the cases of "active" optokinetic nystagmus, the slow component of this type of nystagmus is not a true tracking movement, in which volition is an essential feature but rather it is the progression of the images of the stripes across the retina which appears to provoke the nystagmus That this contribution from the peripheral retina is indeed a very potent stimulus may be judged from the fact that when foveal vision is absent as in patients with central scotomata the optokinetic response may be considerably enhanced (Hood, 1967) These observations have an important bearing upon

CONGENITAL



VESTIBULAR



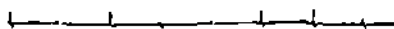


*Fig 3* Spontaneous nystagmus of congenital and vestibular origin

the current interest being taken in the powerful illusory perception of self rotation evoked by an encircling optokinetic drum Dichgans & Brandt (1972) have studied this at length and shown that the illusion of movement induced in a stationary subject by means of an optokinetic drum is indistinguishable from rotation of the subject himself and this they take as evidence of "neuronal vestibular convergence with a relatively long summation time for the visual input and a long time constant of decay after stimulation" Here again it can be shown that the illusion is heavily dependant upon image motion across the re ina Thus in the first place the illusion takes longer to develop and is much less vivid in

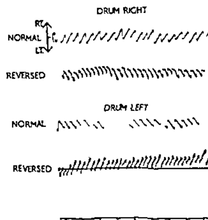



*Fig 4* Reversed optokinetic nystagmus in subject with congenital nystagmus Normal responses to the same stimuli are included for comparison

the "active" form of optokinetic nystagmus than in the "passive" Furthermore, if the subject is asked to fixate upon a stationary target placed close to the periphery of the moving optokinetic drum the illusion of turn ing is immediate and very considerably enhanced despite the fact that the act of optic fixation completely inhibits any nystagmic response This undoubtedly results from the fact that in this situation since the eyes are stationary, retinal image movement is maximal Precisely the same reasoning may be invoked by way of explanation of our repeated observation that patients presenting with a directional preponderance of optokinetic nystagmus consistently report that the illusion of movement is greatest with drum rotation evoking nystagmus in the direction in which the slow component is deficient, that is to say, the direction in which there exists the greatest difference between drum velocity and slow component velocity

The above considerations have special rele vance to the interpretation of certain unusual phenomena encountered in two groups of patients with particular ocular derangements

The first group was concerned for the most part with patients presenting with so-called congenital nystagmus The pathology of this condition is obscure its only manifestation being the presence of spontaneous, rapid and often large amplitude nystagmic movements of the eyes which give rise to relatively little incapacity Although the character of the nys tagmus may on occasions take on a vestibular appearance with a fast component in one direction and a slow component in the other it is more usually described as being pendular, having the form shown in Fig 3 in which for comparison is shown a tracing of true vestibular nystagmus

One of the unusual features commonly held to be pathognomonic of congenital nystagmus is that such patients not infrequently exhibit a *paradoxical reversed optokinetic response*, that is to say with drum rotation say to the right which would normally evoke a nystagmus

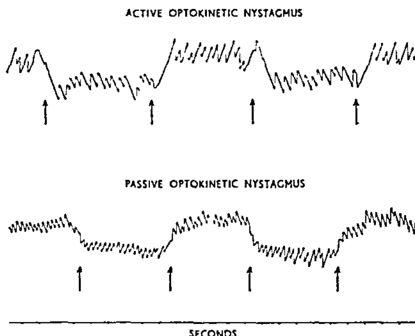



*Fig 2* Nystagmus tracings obtained with a subject following the stripes of the drum (Active optokinetic nystagmus) and gazing passively at the drum (Passive optokinetic nystagmus) Arrows indicate reversal of drum direction Note deviation of eyes in the direction of the slow component of the nystagmus in the case of active optokinetic nystagmus and in the direction of the fast component in the case of passive optokinetic nystagmus.

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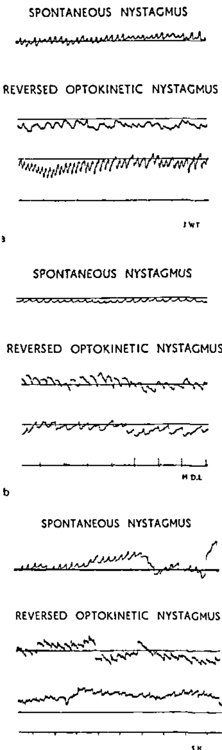



*Fig 6* Spontaneous nystagmus and reversed optokinetic response in patients with (*a*) Miner s nystagmus *b*) Cerebellar encephalopathy (*c*) Multiple sclerosis

velocity is at times considerably slower than normal

As will be seen from the tracings of spontaneous nystagmus in each case although they are manifestly not vestibular in character neither are they truly pendular and in consequence such irregularities as exist will tend to persist in the course of their transformation by the optokinetic stimulus Vagaries of form of this kind are of course to be expected in accordance with the hypothesis put forward, indeed their existence can be said to lend added support to it

The second group of subjects three in all, had complete unilateral ophthalmoplegia Tracings of the optokinetic responses of these subjects were obtained by means of simultaneous recordings from each eye, first with the normal eye covered and the paretic eye uncovered and secondly with the paretic eye covered and the normal eye uncovered Although under neither of these conditions were we able to detect any movement of the paretic eye exposure of this eye to the optokinetic stimulus consistently induced a vigorous indirect response in the covered normal eye Similar findings have previously been reported by Ohm (1926) Kornmuller (1931) and others

Recordings from the normal eye under conditions of direct and indirect stimulation are

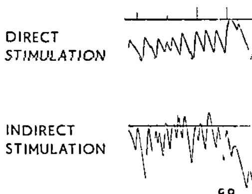



*Fig 7* Optokinetic responses from subject with unilateral ophthalmoplegia Stimulus 20°/sec [1] Direct stimulation—response from normal eye paretic eye covered Indirect stimulation—response from normal eye induced from stimulation of paretic eye normal eye covered

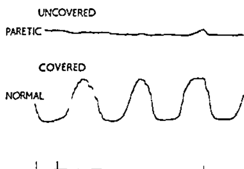



*Fig 8* Following movements induced in normal eye of subject with unilateral ophthalmoplegia from stimulation of paretic eye, normal eye being covered Note absence of movement in tracing from paretic eye

shown in Fig 7 and it will be seen that in this instance the indirectly induced response is enhanced in respect of both amplitude and frequency However, although in all three subjects we never failed to elicit an indirectly induced nystagmus, enhancement was by no means a consistent finding in any subject Nevertheless these findings do seem to provide ample confirmation of our contention that it is the passage of the images of the stripes across the peripheral retina which is the stimulus largely responsible for the optokinetic response Furthermore as might be expected, the reported illusion of turning was always greater when derived from the paretic eye

The tracings shown in Fig 8 are from one of the three patients but involve a different visual stimulus Again, the normal eye was covered and the paretic eye uncovered To the front of the paretic eye a target was moved smoothly from side to side over an arc subtending some 20 degrees

The result as can be seen, is that despite the fact that no movement occurred in the paretic eye the moving stimulus gave rise to a simultaneous smooth pendular movement of the covered normal eye Subsequent measurements have shown the extent of this movement to be 60 degrees considerably exceeding that of the moving target

This finding is difficult to explain in terms of current notions and would appear to have significant implications in the theory underlying ocular following movements It is conventionally held that the only situation in which smooth following movements of the eyes can be elicited is when the image of a moving target is fixed and held upon the fovea To this end it is necessary to suppose that a variety of neural feedback mechanisms are involved aimed at maintaining clear foveal vision upon the moving target

The findings presented in this paper, while they do not contravene these principles suggest that other mechanisms may be involved and in particular that the role of the peripheral retina warrants further consideration

## ZUSAMMENFASSUNG

Wie gezeigt werden konnte bestehen bestimmte signifikante Unterschiede in dem Charakter der optokinetischen Reaktion, und zwar je nachdem ob die Versuchsperson ihren Blick , aktiv oder , passiv auf eine sich drehende Trommel festigt auf die schmale weisse Langsstreifen aufgebracht sind Es ist daher zu vermuten dass den beiden Reaktionen unterschiedliche nervose Mechanismen unterliegen Die Bewegung des Bildes uber die Netzhaut scheint der Reiz zu sein der die , passive Variante auslost und weiterhin ist sie weitgehend die Ursache fur die Illusionseindrucke bei Rotation der Versuchsperson Den gleichen Mechanismus kann man sich auch als Erklarung des Phanomens des umgekehrten optokinetischen Nystagmus vorstellen Das Auftreten dieses Phanomens sowohl bei Patienten mit angeborenem Nystagmus wie auch bei Patienten mit nicht angeborenem Nystagmus stutzt diese Erklarung Die Augenbewegungen die bei Patienten mit einseitiger Ophthalmoplegie durch Reiz des paretischen Auges am normalen Auge ausgelost werden sind eine weitere Stutze fur die Auffassung dass das periphere Sehvermogen zur Kontrolle der normalen Augenbewegungen beitragt

## REFERENCES

Borries G V T 1926 *Fixation and nystagmus* Copenhagen Linds

Brucher J M 1964 *L aire oculogyre frontale du singe* Editions Arscia S A

Dichgans J & Brandt Th 1972 Visual vestibular interaction and motion perception in cerebral control of eye movements and motion perception *Bibl Ophthal 82* 327

Dix M R & Hood J D 1971 Further observations upon the neurolog cal mechanism of opto-

kinetic nystagmus *Acta Otolaryng* (Stockh) *71*, 217

Hood J D 1967 Observations upon the neurological mechanism of optokinetic nystagmus with especial reference to the contribution of peripheral vision *Acta Otolaryng* (Stockh) *63* 208

Kestenbaum A 1948 *Clinical methods of neuro-ophthalmologic examination* William Heinemann London

Kornmuller A E 1931 Eine experimentelle Anasthesie der ausseren Augenmuskeln am Menschen und ihre Auswirkungen *J Psychol Neurol Lp*, *41* 354

Ohm J 1926 Ist der optische Drehnystagmus von einem unbeweglichen Auge auslosbar? *Klin Mbl Augenheilk 21* 354

Rademaker G G J & Ter Braak, J W G 1948 On the central mechanism of some optic reactions *Brain 71* 48

Westheimer, G 1954 Eye movement responses to a horizontally moving visual stimulus *A M A Arch Ophthal 52* 932

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# INTERACTION BETWEEN THE UTRICLES AND THE VERTICAL SEMICIRCULAR CANALS

## V Bilateral Selective Sectioning of Anterior or Posterior Ampullar Nerves or Unilateral Selective Sectioning of the Two Vertical Ampullar Nerves followed by Tilting around their Bitemporal or Longitudinal Axis

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(Received May 8 1973)

*Abstract* Selective bilateral sectioning of the anterior ampullar nerves was performed in 29 cats This procedure resulted in a spontaneous nystagmus upwards If the animals were tilted around their bitemporal axis nose downwards nystagmus was inhibited Tilting nose upwards increased the nystagmus frequency Selective bilateral sectioning of the posterior ampullar nerves caused a spontaneous nystagmus downwards Tilting nose downwards increased the nystagmus whereas tilting in the opposite direction caused its inhibition Selective unilateral sectioning of the anterior and posterior ampullar nerves was followed by a rotatory nystagmus which increased if the animals were tilted around their longitudinal axis towards the non-operated ear and which was totally inhibited if they were tilted in the opposite direction The cause of this phenomenon is discussed

In a series of articles the present authors (1973 *a b c*) have established that the utricles and the horizontal semicircular canals cooperate very intimately An increased input from the utricular area that causes horizontal eye movements, facilitates nystagmus released from the ipsilateral horizontal semicircular canal, but simultaneously inhibits nystagmus released from the contralateral horizontal canal From this it has been concluded that the input from the utricle goes to the same oculomotor muscles as those receiving impulses for the slow phase of horizontal nystagmus There is either a summation or a subtraction of activity, depending on whether the ipsilateral or contralateral utricle is activated Earlier investigations (Fluur & Mellstrom, 1970) have shown that electrical stimulation of certain areas on the utricular surface causes distinct vertical eye movements Consequently, because there is every reason to suppose that cooperation exists also between the utricles and the vertical semicircular canals, we have found it a matter of great interest to explore this problem whose existence has hitherto remained unknown

## MATERIAL AND METHOD

Twenty nine cats, divided into three groups, were used for the experiments

1 Selective bilateral sectioning of the anterior ampullar nerves followed by tilting around the bitemporal axis of the cat (13 cases)

2 Selective bilateral sectioning of the posterior ampullar nerves, followed by tilting around the bitemporal axis of the cat (13 cases)

3 Selective unilateral sectioning of the two vertical ampullar nerves, followed by tilting

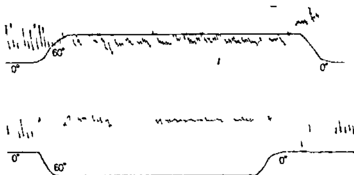



*Fig 1* Consecutive curves from a cat after selective bilateral sectioning of the anterior ampullar nerves followed by tilting nose upwards and downwards The upper curve shows electronystagmography and the lower curve the tilting of the table Tilting nose upwards, curve upwards tilting nose downwards curve downwards

around the longitudinal axis of the cat (3 cases)

The cats were first anesthetized with ether, by a so-called Ayer-system, which is employed in pediatric anesthesia After completing the operation they were given Ketamine (Parke Davis), a non barbituratic anesthetic, characterized by pronounced analgetic properties but with minimal effects on the reticular formation in order to maintain a superficial level of anesthesia, without the animals moving or feeling pain, but with the reflex activity, including nystagmus remaining fully developed

For sectioning of the anterior ampullar nerves the approach was the same as that for the horizontal ampullar nerve (Fluur & Siegborn, 1973) In order to expose the posterior ampullar nerve a retroauricular incision was made, and the bulla was opened Immediately behind the round window a fenestration was performed and the posterior ampullar nerve was exposed and cut Great care must be taken not to damage the horizontal canal immediately above the ampulla, otherwise the cat developed horizontal nystagmus

The eye movements were recorded partly by direct visual ocular inspection of the cat's eye, and partly by electronystagmography, with the electrodes above and below one eye, when recording vertical nystagmus Because rotatory nystagmus cannot be recorded by any electrical method only visual inspection was applied in these experiments

The animals were stimulated by tilting them 30 degrees or 60 degrees around their bitemporal or longitudinal axis

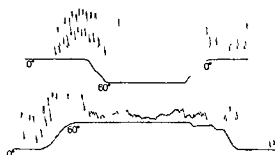
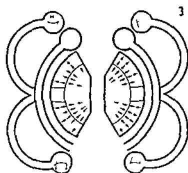


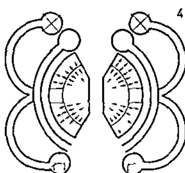

*Fig 2* Consecutive curves from a cat after bilateral sectioning of the posterior ampullar nerves followed by tilting nose downwards and upwards The upper curve shows electronystagmography and the lower curve the tilting of the table Tilting nose downwards curve downwards tilting nose upwards curve upwards

## RESULTS

After bilateral, selective sectioning of the anterior ampullar nerves all the 13 cats showed vertical nystagmus upwards with 4–11 beats/10 sec In all these cats tilting nose downwards resulted in inhibition of nystagmus, which, in 11 cases, was total (Fig 1) In 2 cats the frequency decreased from 5 to 2 beats/sec If, on the other hand, the cats were tilted nose upwards, the spontaneous nystagmus increased in frequency by 1–17 beats/10 sec in 12 cats and remained more or less unchanged in one cat (Fig 1)

All 13 cats, where bilateral sectioning of the posterior ampullar nerve was performed, had vertical nystagmus downwards, with 8–21 beats/10 sec If they were tilted nose down





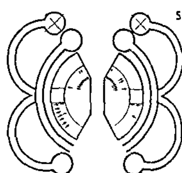



*Fig 3* Schematic diagram of the utricles and vertical semicircular canals in resting position The arrows indicate the orientation of the hair cells i e, the direction in which they increase the discharge frequency

*Fig 4* Schematic diagram of the utricles and vertical semicircular canals after bilateral selective sectioning of the anterior ampullar nerves and when the animal is in resting position Arrow indication same as in Fig 3 The number of arrows symbolizes discharge frequency

*Fig 5* Schematic diagram of the utricles and vertical semicircular canals after bilateral selective sectioning of the anterior ampullar nerves and when the animal is tilted nose downwards Arrow indication same as in Fig 3 The number of arrows symbolizes discharge frequency

wards, this caused definite increase in nystagmus frequency, by 6–27 beats/10 sec in 10 cases (Fig 2) In another 3 cats there was no substantial alteration in the nystagmus If, instead, they were tilted nose upwards, nystagmus disappeared entirely in 11 cats, in 2 other cats nystagmus frequency decreased by about 6 beats/10 sec, but no total inhibition occurred (Fig 2)

In both these series it occurred of course, that one and the same cat could show both a decrease in nystagmus and total inhibition, depending on its degree of the wakefulness of the cat But in general, reaction was always according to the principles involved In some of the animals inhibition occurred immediately, in others it took about 5–10 sec

Selective sectioning of the anterior and posterior ampullar nerves on the left side induced in the 3 cats on counterclockwise rotatory nystagmus (seen from the observer) If these cats were tilted around their longitudinal axis towards the non-operated ear, the nystagmus increased in frequency, when, however, they were tilted towards the operated ear the nystagmus disappeared completely

## DISCUSSION

The view expressed in earlier investigations (Fluur & Siegborn, 1973 *a*, *b*, *c*) concerning the interplay between the utricles and the horizontal semicircular canals, namely that the utricular influence on the horizontal eye movements is added to the slow phase of nystagmus, has proved to be fully valid also for the vertical semicircular canals regarding both vertical and rotatory nystagmus

Earlier publications regarding the finer anatomical orientation of the sensory cells of the otolith organs (Lowenstein & Wersall, 1959) and the neurophysiological experiments concerning the utricular influence on the oculomotor activity (Fluur & Mellstrom, 1970) make it possible today, without theorizing, to explain the cause of the results obtained in the present experiments

The electrical activity of the vertical semicircular canals and that of the 2 utricles are, in the resting position, completely balanced mutually (Fig 3) After selective sectioning of the anterior ampullar nerves the input from these semicircular canals disappears, and the

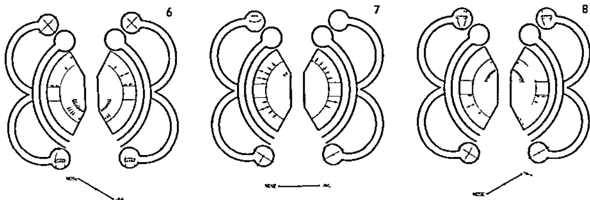



*Fig 6* Schematic diagram of the utricles and vertical semicircular canals after bilateral selective sectioning of the anterior ampullar nerves and when the animal is tilted nose upwards Arrow indication same as in Fig 3 The number of arrows symbolizes discharge frequency

*Fig 7* Schematic diagram of the utricles and vertical semicircular canals after bilateral selective sectioning of the posterior ampullar nerves and when the animal is in resting position Arrow indication same as in Fig 3 The number of arrows symbolizes discharge frequency

*Fig 8* Schematic diagram of the utricles and vertical semicircular canals after bilateral selective sectioning of the posterior ampullar nerves and when the animal is tilted downwards Arrow indication same as in Fig 3 The number of arrows symbolizes discharge frequency

cat experiences a vertical nystagmus upwards with the slow phase downwards (Fig 4) If the cat is now tilted nose downwards (Fig 5) total inhibition develops in those sensory cells on the utricular surface which induce eye deviations downwards at the same time as an increased input is being built up in those areas which cause elevation of the eyes The outcome is a subtraction of input from or antagonism between the utricles and the posterior canals to the slow phase downwards This inhibits the input to the oculomotor depressors and nystagmus disappears

If on the other hand the cat is tilted nose upwards (Fig 6), the input increases in those utricular areas causing ocular depression and simultaneously the activity is inhibited in those areas causing elevation of the eyes The outcome is a summation of input from or synergism between the utricles and the posterior canals to the slow phase downwards which increases the intensity of nystagmus upwards

If however, the posterior ampullar nerves are cut the input from these semicircular canals disappears and the animal has vertical nystagmus downwards with the slow phase upwards (Fig 7) Tilting nose downwards (Fig 8) inhibits those utricular areas causing depression of the eyes and the activity increases in those areas giving elevation The outcome is an addition or synergism of activities from the anterior canals and the utricles to those oculomotor muscles giving elevation and this increases the nystagmus intensity downwards

If the cat is tilted nose upwards (Fig 9) quite the contrary situation develops and the nystagmus downwards is completely suppressed

If finally a selective unilateral sectioning of the anterior and posterior ampullar nerves is performed the balance of activity is disturbed in favour of the contralateral vertical canals which induce a counterclockwise rotatory nystagmus with the slow phase beating towards the operated ear (upper eye pool) (Fig 10) If the cat is now tilted around the longitudinal axis towards the non-operated side (Fig 11) increased activity accumulates in the lateral part of the utricle which faces upwards and inhibition develops simultaneously in the medial parts In the utricle facing downwards the situation is the contrary This induces an increased input into those oculomotor muscles

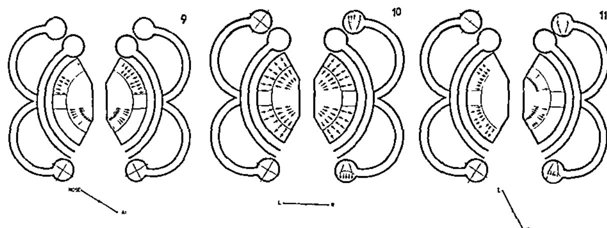



*Fig 9* Schematic diagram of the utricles and vertical semicircular canals, after bilateral selective sectioning of the posterior ampullar nerves, and when the animal is tilted nose upwards Arrow indication same as in Fig 3 The number of arrows symbolizes discharge frequency

*Fig 10* Schematic diagram of the utricles and vertical semicircular canals, after unilateral selective sectioning of the left anterior and posterior ampullar nerves, and when the animal is in resting position Arrow indication same as in Fig 3 The number of arrows symbolizes discharge frequency

*Fig 11* Schematic diagram of the utricles and horizontal semicircular canals, after unilateral selective sectioning of the left anterior and posterior ampullar nerves, and when the animal is tilted towards the right Arrow indication same as in Fig 3 The number of arrows symbolizes discharge frequency

which cause deviation of the upper eye pool in the direction of the operated ear. The result is again a summation or synergism of input from the intact, vertical canals and the utricles, which increases the intensity of nystagmus

If, however, the animal is tilted in the other direction, the result is entirely the opposite (Fig 12)

Consequently, it has been shown that the labyrinth is a very complicated computer, where every alteration in activity in anyone of its separate sensory organs immediately in-

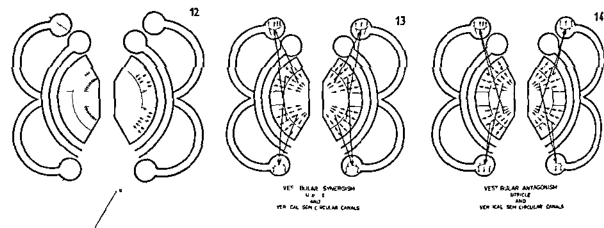



*Fig 12* Schematic diagram of the utricles and vertical semicircular canals after unilateral selective sectioning of the left anterior and posterior ampullar nerves, and when the animal is tilted to the left Arrow indication same as in Fig 3 The number of arrows indicates discharge frequency

*Fig 13* Schematic diagram showing vestibular synergism between the utricles and the vertical, semicircular canals

*Fig 14* Schematic diagram showing vestibular antagonism between the utricles and the vertical semicircular canals

fluences the interplay between the other organs, so that an alteration occurs in the activity of the effector organs—in this case, the oculomotor muscles Consequently, the vertical canals and the utricles also form one system of synergetically functioning sensory organs (Fig 13) and another system, which functions antagonistically (Fig 14)

## ZUSAMMENFASSUNG

Bei 29 Katzen wurde eine bilaterale, selektive Abschneidung des vorderen vertikalen N ampullaris vorgenommen Dies ergab einen vertikalen Nystagmus nach oben Eine Kippung in der bitemporalen Achse, mit der Nase nach unten, lies den Nystagmus verschwinden Eine Kippung mit der Nase nach oben ergab eine Steigerung der Nystagmusgeschwindigkeit Selektive, bilaterale Abschneidung des hinteren vertikalen N ampullaris ergab einen vertikalen Nystagmus nach unten Eine Kippung mit der Nase nach unten steigerte die Geschwindigkeit des Nystagmus Eine Kippung in die andere Richtung inhibierte den Nystagmus Wurde eine selektive, unilaterale Abschneidung des vorderen und hinteren N ampullaris vorgenommen, so stellte sich ein rotatorischer Nystagmus ein, der sich steigerte, als die Tiere in der longitudinalen Achse nach dem nicht operierten Ohrhin gekippt wurden und der bei einer Kippung in die andere Richtung total verschwand Die Grunde dafur wurden diskutiert

## REFERENCES

Fluur, E & Mellstrom, A 1970 Utricular stimulation and oculomotor reactions *Laryngoscope 80*, 1701

Fluur, E & Siegborn, J 1973 *a* Interaction between the utricles and the horizontal semicircular canals I Unilateral selective sectioning of the horizontal ampullar nerve followed by tilting around the longitudinal axis *Acta Otolaryng* (Stockh ) *75*, 17

— 1973 *b* Interaction between the utricles and the horizontal semicircular canals II Unilateral selective sectioning of the horizontal ampullar and the utricular nerve, followed by tilting around the longitudinal axis *Acta Otolaryng* (Stockh ) *75*, 393

— 1973 *c* Interaction between the utricles and the horizontal semicircular canals III Sectioning of the horizontal ampullar nerve on one side and of the utricular nerve on the other, followed by tilting around the longitudinal axis *Acta Otolaryng* (Stockh ) *75*, 485

Löwenstein, O & Wersall, J 1959 A functional interpretation of the electron microscopic structure of the sensory hairs in the cristae of the Elasmobranch *Raja Clavata* in terms of directional sensitivity *Nature 184*, 1807

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# EYE MOVEMENTS AS A FUNCTION OF ACTIVE HEADTURNINGS

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*Abstract* Eye movements caused by turnings of the head present a very stable pattern with the eyes directed in the same direction as the headturnings The initial deviation the maximum deviation and the resting deviation of the eyes have been calculated as a function of the velocity of the head This study is meant to form a basis for a future analysis of pathological cases in which other patterns of eye movements are found

Eye movements caused by passive turnings of the head and body have been the topic of an extensive literature (Barany, 1906, Jongkees & Philipszoon, 1964, Aschan et al, 1956, Henriksson et al, 1972) Eye movements caused by passive turnings of the body in relation to the fixed head have been described in numerous papers (Barany, 1907, Magnus, 1924, Biemond, 1939 and 1940, Philipszoon & Bos, 1963, Bos & Philipszoon 1963 Takemori & Suzuki 1971)

Eye movements at active turnings of the head were studied by Guttich (1962) who described the relations between the movements of the head when the subject wanted to fixate a target to the right or left in his field of vision Güttich described rapid deviation of the eyes in the direction of the active turning but made no quantitative estimations Kurosawa (1965) used a linear accelerometer and electronystagmograph to determine the relation in time with the eye movements and head rotation and found different patterns in these relations These patterns could be related to age but also to vestibular asymmetries such as preponderance

Bartz (1966) studied with objective recordings the movement of the eyes and head in response to a visual target to the right and left He studied quantitatively the time relation between the head and eye movements He found a rapid deviation of the eyes in the direction of the target largely preceding the movement of the head After this rapid deviation of the eyes, a slow returning of the eyes was found to take place compensating the head movements Some of this slow returning of the eyes was found to take place after the end of the head movements

These studies of eye movements at active headturnings were all made in test situations in which the subject was actively engaged in looking towards some target located in the periphery of or outside his field of vision

A different test situation is the one in which the test subject is actively turning his head without any special intention to direct his gaze for any kind of observing A quantitative study of the involuntary eye movements appearing at such situations with active headturnings (concomitant eye movements) does not seem to have been the topic of any previous paper This together with the fact that so many patients complaining of dizziness and vertigo claim that these difficulties arise or increase at active head turnings initiated the present study The aim of this paper will therefore be to map out such concomitant patterns of eye movements at headturnings in normals

## METHOD

The turnings of the head were recorded by means of a potentiometer fixed on top of th

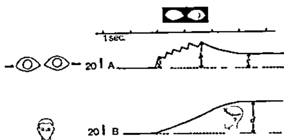



*Fig 1* Normal pattern of eye movements at turning of the head (*a*) Initial deviation of the eyes (*b*) Maximum deviation of the eyes (*c*) Resting deviation of the eyes (*d*) Resting deviation of the head

head as previously described (Lundgren et al, 1969) The movements of the eyes were recorded with a conventional DC-recorder (Henriksson et al, 1972)

## MATERIAL

Six normal subjects, aged 25–27, students of medicine without any previous vestibular complaints, were used as test subjects

### *Procedure of the test*

The test subjects were instructed with the help of a tape recorder There was first given general information about the test and about what was expected from the test subject The test subject was told to turn the head as much as possible without any discomfort

The head was first turned to the right (one part turning), kept in this deviated position for three seconds and then turned back to mid-position After three seconds in mid position a turning to the left took place, a pause for three seconds in this position and then again back to mid position Thus, a complete turning was made up by four part turnings Five such complete turnings to the right and left were done, four different velocities of the headturnings were used On instruction from the tape recorder the subjects were told to turn their heads along with the counting from the recorder

1 One turning in six seconds
2 One turning in four seconds
3 One turning in two seconds
4 One turning in one second

As four different turnings of the head were made (towards the right, back to mid position, towards the left and back to mid-position) at four different velocities, all together sixteen different kinds of headturnings were made As each such turning was repeated five times, from each subject was achieved 80 recordings As the tests were made both in light and darkness each subject presented 160 curves and as six subjects were used, the whole study was based upon 960 recordings of head- and eye movements

The tests were all performed in the afternoon between 3 and 4 p m

### *Definition of parameters*

For each of the sixteen different types of head-turnings averages were calculated with respect to the following parameters

I Initial deviation of the eyes = amplitude of the first fast nystagmus phase (*a* in Fig 1)
II Maximum deviation of the eyes = the maximum angular deviation of the eyes from their mid-position (*b* in Fig 1)
III Total slow phase of all nystagmus beats — the sum of all slow phases achieved at one part turning of the head.

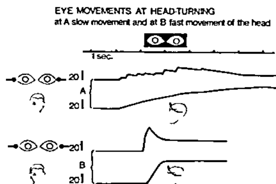
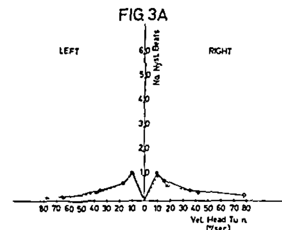
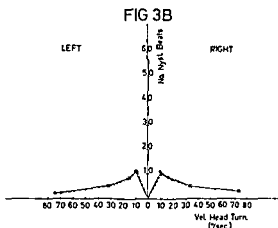
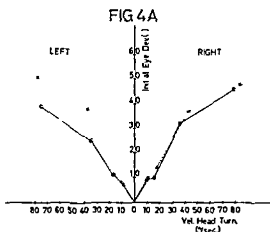
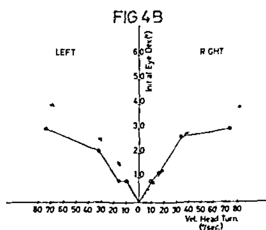



*Fig 2* Simultaneously recorded position of the eyes and turning of the head The two upper curves achieved at a slow turning of the head, two bottom pairs achieved at a rapid turning of the head




*Figs 3–8* Different parameters of eye movements in head turnings as a function of angular velocity of the head (*A*) Values measured in light (B) Values measured in darkness o—o = Turnings of the head from mid position towards right and left ×---× = Returning of the head towards mid position

IV Average velocity of the eyes in the slow phases =

$$\frac{\text{Total amplitude of all slow phases}}{\text{Total time for slow phases}}\ °/\text{sec}$$

V Resting deviation of the eyes – deviation of the eyes at the very end of the last slow phase (*c* in Fig 1)

VI The number of nystagmus beats during turning of the head

VII Angular velocity of the headturnings =

$$\frac{\text{Maximum deviation of the head}}{\text{Time for head turning}}\ °/\text{sec}$$

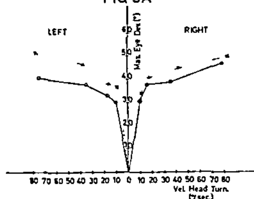
A special interest was directed towards these above mentioned parameters as functions of the velocity of the head

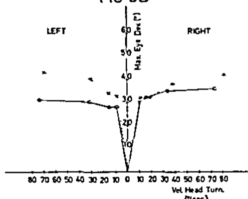
## RESULTS

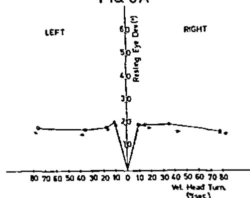
1 All test subjects showed at each turning of the head a deviation of the eyes in the direction of the turning (Fig 1)

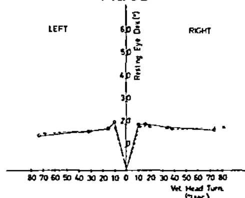
2 The fast headturnings resulted in only one fast phase of eye movements in the direction of turning and one slow phase in opposite direction (Fig 2) When the head was turned more slowly, the eyes performed a number of nystagmus beats in the direction of turning and always with a smaller number at high velocities of the head (Fig 2)

3 At the slow turnings of the head (one turning in six seconds) there was about ten nystagmus beats, while at rapid head movements the




eyes performed only one full nystagmus jerk (Fig 3 A and B)

4 The initial eye deviation increased with the velocity of the head This deviation was almost a linear function of the velocity of the head up to 40°/sec, but seemed at higher velocities to increase more slowly The initial deviation of the eyes is larger in light than in darkness (Fig 4 A and B)

5 The maximum deviation of the eyes was always reached well before the end of the movement of the head and reached higher values at rapid turnings of the head The maximum eye deviation was larger in light than in darkness (Fig 5 A and B)

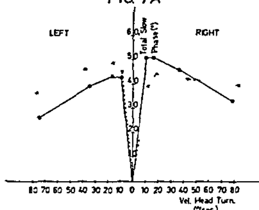
6 During and after the last part of headturning the eyes returned but did not fully reach their mid position The resting deviation, still present when the eyes came to a rest, was only slightly larger after slow than after high velocities of the head (Fig 6 A and B)

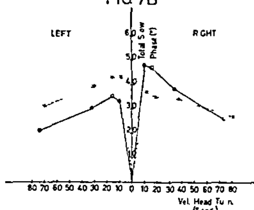
7 The total slow phase amplitudes were found larger at slow turnings of the head than in rapid turnings Further, total slow phase amplitudes were also larger in light than in darkness (Fig 7 A and B)

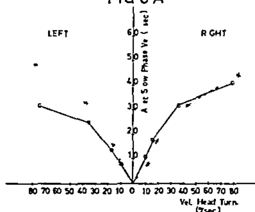
8 The average slow phase velocity increased apparently as a linear function of movements of the head up to about 40°/sec When the head was turned at higher velocities, the eyes seemed to lag behind the head In darkness the velocity of the eyes do not even at slow velocity of the head correspond to the movement of the head (Fig 8 A and B)

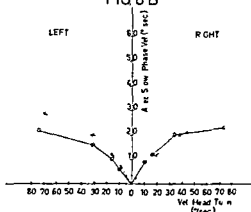
## DISCUSSION

The results presented here indicate a very constant pattern The eyes always deviated in the




direction of the turning of the head An anticipated fixation of the eyes straight ahead was never found It should also be pointed out that the test subjects only were instructed to turn your head

Among possible factors responsible for the constant pattern of eye movements the following three mechanisms may be considered

1 It has been shown that a torsion of the neck (also without turning of the head) causes a compensating deviation of the eyes and may also cause nystagmus (Philipszoon & Bos 1963 Takemori & Suzuki 1971) These eye movements are thought to be initiated by proprioceptive afferent impulses from neck muscles It must therefore at least be discussed if the concomitant eye movements described here are to some extent released or at least facilitated by afferent activity in the neck muscles

2 Another mechanism causing a deviation of the eyes in the direction of the headturning would be an anticompensatory movement described by Melvill Jones (1964) and also later by Lundgren et al (1969) and by Goto et al (1968) Such a deviation seems to be provoked by intensive vestibular stimulation causing during the first part of the nystagmus reaction a displacement of the eyes always in the direction of the turning of the head As however the concomitant eye movements show a quite normal pattern also in patients with no labyrinthine reactions the vestibular stimulation in the headturning could not possibly be considered responsible for the concomitant eye movements

3 Impulses causing movements of the head may also be combined with parallel impulses reaching eyemotor nuclei causing meaningful deviation of the eyes in the same direction as

the head This linkage between quite different groups of muscles must be a common phenomenon in performance of frequently used complex movements and may be acquired in extra uterine life Its stability may be an expression of its meaningfulness It is interesting to mention in this connection that in newborn children such linkage is not yet developed In these very young children the eyes will deviate opposite the direction of the movements of the head (Doll's phenomenon) This deviation opposite to the movements of the head anyhow takes place at passive turnings of the head (Bickerstaff, 1963) The importance of the meaningfulness of the concomitant eye movements may be stressed by the fact that in blind persons there are less or no such concomitant eye movements

Irrespective of what the mechanism may be for this concomitant eye movements, a thorough knowledge of these eye movements in normal conditions must form a basis for an understanding of different patterns in pathological conditions

## ZUSAMMENFASSUNG

Durch Kopfdrehungen verursachte Augenbewegungen in Richtung dieser Drehungen folgen einem sehr stabilen Schema Die Initial-, Maximal- und Ruhewerte fur die Deviation der Augen sind als Funktion der Winkelgeschwindigkeit des Kopfes berechnet worden Die vorliegende Arbeit bildet den Ausgangspunkt fur kommende Studien pathologischer Falle, bei denen abweichende Arten der Augenbewegungen festgestellt werden konnen

## REFERENCES

Aschan, G, Bergstedt, M & Stahle, J 1956 Nystagmography, recording of nystagmus in clinical neuro-otological examination *Acta Otolaryng* (Stockh), Suppl *129*

Aschan, G & Hugosson, R B 1966 Vestibular symptoms provoked by head and neck rotation after bilateral carotid ligation *Acta Otolaryng* (Stockh) *61*, 49

Barany, R 1906 Untersuchung uber den vom Vestibularapparat des Ohres reflektierenden ausgelosten rhytmischen Nystagmus und seine Begleiterscheinungen. *Mschr Ohrenheilk 40*, 193

— 1907 Augenbewegungen durch Thoraxbewegungen ausgelost *Zbl Physiol 20*, 198

Bartz, A E 1966 Eye and head movements in peripheral vision Nature of compensatory eye movements *Science 152*, 3729

Bickerstaff, E R 1963 *Neurological examination in clinical practice* Billing & Sons, Guilford and London

Biemond, A 1939 On a new form of experimental position nystagmus in the rabbit and its clinical value *Proc Kon Ned Akad Wet* (Amsterdam) *42*, 370

— 1940 Further observations about the cervical form of position nystagmus and its anatomical basis *Proc Kon Ned Akad Wet* (Amsterdam) *43*, 2

Bos, J H & Philipszoon, A J 1963 Some forms of nystagmus provoked by stimuli other than accelerations *Pract Otorhinolaryng* (Basel) *25*, 108

Goto, K., Tokumasu, K & Cohen, B 1968 Return eye movements, saccadic movements, and the quick phase of nystagmus *Acta Otolaryng* (Stockh) *65*, 426

Guttich, H 1962 Zur Physiologie von Bogengangs- und Otolithenapparat *Mschr Ohrenheilk 96*, 490

Henriksson, N G, Rubin, W, Pfaltz, C R & Torok, N 1972 *A Synopsis of the Vestibular System* Monograph Sandoz, Schweiz

Jongkees, L B W & Philipszoon, A J 1964 Electronystagmography *Acta Otolaryng* (Stockh), Suppl *189*

Kurosawa, T 1965 Studies on physiological relationship between the head and eye movements in case of active head rotation Proposal of a newly devised rotation test *Pract Otolaryng* (Kyoto) *58*, 353

Lundgren, A, Tibbling, L & Henriksson, N G 1969 DC-determined displacement of the nystagmus beat in rotatory tests *Pract Otorhinolaryng 31*, 54

Magnus, R 1924 *Korperstellung* Springer, Berlin

Melvill Jones, G 1964 Predominance of anti-compensatory oculomotor response during rapid head rotation *Aerospace Med* 965

Philipszoon, A J & Bos, J H 1963 Neck torsion nystagmus *Pract Otorhinolaryng* (Basel) *25*, 219

Takemori, S & Suzuki, J-I 1971 Eye deviations from neck torsion in humans *Ann Otol 80*, 3

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# THE LOCALIZATION OF VESTIBULAR EFFERENT NEURONS IN THE KITTEN WITH HORSERADISH PEROXIDASE

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*Abstract* The location of efferent vestibular neurons in the brainstem of newborn kittens was determined by means of horseradish peroxidase used as a tracer of retrograde protein transport Two to three milligrams of horseradish peroxidase were injected into the vestibule of 8 kittens After survival for 24 hours, the animals were fixed by perfusion and the brainstems sectioned Stains for peroxidase demonstrated the labelled neurons lateral to the abducens nucleus bilaterally

An efferent component of the vestibular nerve was first demonstrated in 1958 (Rasmussen & Gacek) and 1960 (Gacek) with axonal de- [illegible] techniques following lesions in the [illegible] of the cat Since then the efferent vestibular pathway has also been demonstrated with histochemical techniques utilizing the property of acetylcholinesterase localization in efferent nerves and terminals (Gacek et al, 1965, Gacek, 1967) Furthermore, the terminals of the efferent pathway have been described in detail with the electron microscope (Smith & Rasmussen, 1967) Over the past 15 years more than 100 lesions in the vestibular nuclei and cerebellum have been produced and the resulting degeneration in the vestibular nerve branches studied in order to determine the precise location of the origin of the vestibular efferent pathway Utilizing this technique the cerebellum as well as the superior vestibular nucleus, medial vestibular nucleus and dorsal part of the lateral vestibular nucleus have been ruled out as a possible source of the efferent vestibular fibers The probable location of the vestibular efferent neurons has been postulated in the vicinity of the ventral division of the lateral vestibular nucleus or possibly the reticular formation (Gacek, 1967) No indication of a contralateral origin to the bundle has been found by this author

The traditional method of determining the cells of origin of a neural pathway is to elicit the retrograde cell reaction following transection of the cell's axon Brodal's (1940) modification of this technique has been utilized in young kittens after unilateral labyrinthectomy but no unequivocal cell changes could be found that would justify location of the vestibular efferent neurons (Gacek, 1964) It is extremely difficult to identify chromatolysis and displacement of the nucleus in very small neurons All available information on the size of the axons of the efferent neurons indicates that these are small sized neurons Rossi & Cortesina (1962) did state on the basis of cell changes and cell loss following vestibular nerve section in the guinea pig that they could demonstrate the origin of the vestibular efferent bundles They found two bundles, one arising from the lateral vestibular nucleus and a more ventral bundle which arose from a

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nucleus called the 'nucleus interpositus" This small nucleus was located between the lateral vestibular nucleus and the descending trigeminal root However, because of the equivocal nature of the cell changes, this origin has not been generally accepted

Recent techniques have shown that exogenous proteins which have been presented to the neuromuscular junction of motor nerves such as the hypoglossal nerve (Kristensson et al, 1971, Sjostrand, 1969) or other motor nerves (Zacks & Saito, 1969), will be taken up by the axons and be carried in retrograde fashion to the cell body over a period of 10–24 hours Protein tracers that can be used to demonstrate this retrograde transport are Evans Blue albumin and horseradish peroxidase The tracer can then be demonstrated with staining techniques (Graham & Karnovsky, 1966) in the cytoplasm of the cell bodies located within the central nervous system This property appears to be age dependent and is more demonstrable in the newborn animal (Kristensson & Olsson, 1971)

In the present study newborn kittens were used to demonstrate the location of the cells of the vestibular efferent system in the brain stem after injection of horseradish peroxidase into the vestibule of the inner ear Following a survival time of 24 hours, the animals were sacrificed by perfusion, the brainstems sectioned and stained according to the method of Graham & Karnovsky (1966) The cells of the vestibular efferent system were selectively demonstrated with horseradish peroxidase in an area between the abducens nucleus and the ventral division of the lateral vestibular nucleus bilaterally

## MATERIAL AND METHODS

Eight newborn kittens of age 1 day or less were used as the experimental animal The animals were anesthetized with intraperitoneal Nembutal (0 5 cc/kg) and the vestibule of the inner ear exposed by two approaches 1) In 4 animals an atticotomy approach was used to approach the oval window after making a skin incision posterior and medial to the right auricle After removal of the incus, the mucoid substance which fills the middle ear space was aspirated and the stapes superstructure removed leaving only the footplate of the stapes in the oval window The footplate was elevated carefully and perilymphatic fluid aspirated Then, with a syringe and a fine gauge needle, 20–30 μl of solution containing 2–3 mg of horseradish peroxidase in Ringer's solution was injected into the vestibule and the footplate replaced The middle ear was then filled with a free muscle graft and the incision closed with sutures 2) In three kittens a ventral approach through the bulla was utilized to approach the vestibule After removal of the cartilagenous covering of the bulla and evacuation of the mucoid contents of the bullar space, the round window niche and membrane were identified and the round window membrane carefully perforated The injecting needle was then directed in a superior direction so that the needle point perforated the most basal end of the cochlea and penetrated the vestibule from a ventral direction The 20-30 μl of horseradish peroxidase solution was then injected into the vestibule and gelfoam was then placed in the round window niche This approach was also utilized because the horseradish peroxidase, when injected through the oval window, was difficult to contain within the vestibule and a certain amount of overflow into the middle ear space had resulted In the ventral approach the reflux of horseradish peroxidase was minimal 3) In one animal the atticotomy approach was utilized and the middle ear exposed The incus was removed and mucoid substance aspirated as in the other animals No opening into the vestibule was produced but the same quantity of horseradish peroxidase was instilled into the middle ear space so as to contact the mucosa and the wound was closed

All 8 animals operated on were allowed to survive for 24 hours Then they were again anesthetized with Nembutal and intracardiac



*Fig 1* Photomicrograph (magnification 1200×) of vestibular efferent neurons labelled with horseradish peroxidase The HRP granules are visible in the cytoplasm of 3 neurons Compare to unlabelled neuron (*arrow*)

perfusion was utilized to sacrifice the animals Following a saline flush with the catheter placed in the left ventricle and an exit incision made in the right auricle the fixative of 4% formaldehyde and 5% glutaraldehyde in a 0 1 M phosphate buffer with a pH of 7 2 was used The calvarium was removed and the entire head immersed for 24–36 hours in the fixative before removing the brainstem and cerebellum from the skull Further fixation was allowed in the fixative of 4% formaldehyde and 5% glutaraldehyde and then frozen sections were cut at 25 microns Sections were then incubated for 10–15 minutes at room temperature in a saturated solution of 3 3-diaminobenzidine The sections were washed in 3 changes of distilled water and then mounted on slides using gelatin adhesive After dehydration to absolute alcohol for 10 minutes the sections were counterstained in 0 1% cresylviolet and differentiated through 95% alcohol After dehydration and clearing the sections were covered and ready for examination Complete sets of serial sections were obtained through the brainstem from rostral to the superior vestibular nucleus to the caudal portion of the brainstem These sections were then carefully evaluated and cells with the rusty red horseradish peroxidase granules (Fig 1) were located on an outline drawing of the section

## RESULTS

The chart in Table I summarizes the three groups of animals described in the method section The 4 animals in which the horseradish peroxidase was injected by an oval window approach to the vestibule all demonstrated uptake of the tracer in small cells which were

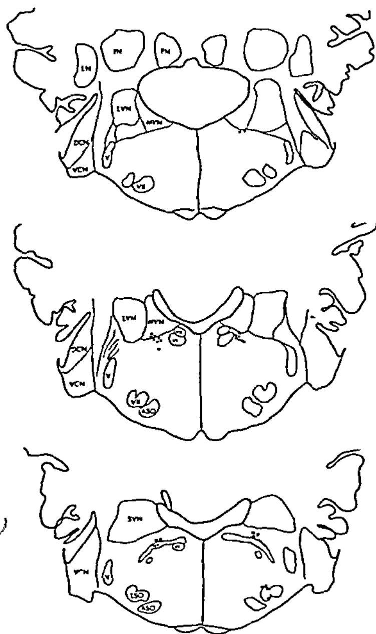
Table I *Chart summarizing the results in experimental and control animals following injection of horseradish peroxidase (HRP)*

| Animal no | Site of injection (HRP) | Uptake in vestibular efferents | Location in brainstem | Number of cells labelled | |
|---|---|---|---|---|---|
| | | | | Ipsilat | Contralat |
| K 1-020273 | Vestibule (O W ) | Very good (3 + ) | Small cells lateral to VI nucleus | 145 | 58 |
| K 2-020273 | Vestibule (O W ) | Good (2 + ) | Same as above | 72 | 32 |
| K 1-022673 | Vestibule (O W ) | Good (2 + ) | Same as above | 70 | 23 |
| K-1-031273 | Vestibule (O W ) | Weak (1 + ) | Same as above | 7 | 3 |
| K 1-022673 | Vestibule (R W ) | None | Diffuse in surface *cells of brainstem* | — | — |
| K-2-031273 | Vestibule (R W ) | Strong (4 + ) | Small cells lat to VI nucleus | 174 | 151 |
| K 1-040273 | Vestibule (R W ) bilat | None | Diffuse in surface cells of brainstem | — | — |
| K 3-031273 | Middle ear | None | — | — | — |

grouped lateral to the abducens nucleus and medial ventral to the lateral vestibular nucleus This localization of cells with the protein tracer occurred bilaterally in all cases However, the intensity of uptake in these cells varied between animals In animal K 1 020273, there were 145 cells ipsilateral to the injection side and 58 on the contralateral side Although the 3 remaining animals demonstrated a lesser intensity of uptake and therefore fewer cells that were countable, it was evident that the number of cells ipsilaterally was greater than contralaterally in all three cases

The second group of 3 animals, in which the injection was performed through the hook portion of the basal turn of the cochlea into the *vestibule*, gave a different experience This method of approach to the vestibule was selected *in an attempt* to maintain a better concentration of horseradish peroxidase within the vestibule In the group of animals where the tracer was injected through the oval window after elevating the stapes footplate, considerable peroxidase overflowed into the middle ear space and was difficult to contain within the vestibule even with soft tissue plugging of the oval window niche In two animals (K-1-022673 and K-1-040273) the peroxidase was not taken up within any cells in the vestibular nuclei but became diffusely located in the surface cells of the ependyma of the brainstem indicating that the injection had been into the cerebrospinal fluid space It became clear that with this approach the peroxidase *was sometimes injected into the internal auditory* canal and the modiolus However, in one animal (K-2-031273) the injection into the vestibule was successful and the horseradish peroxidase was contained entirely within the vestibule with no leakage into the cerebrospinal fluid space In this animal the uptake was stronger than in *any of the animals in the* first group This was evidenced by a greater density of tracer within the cells and in a greater number of cells contralaterally than were identified by the oval window approach Cell counts in this animal indicated there were 174 cells ipsilaterally as compared with 151 contralaterally The location of labelled cells in this animal is shown in Fig 2 where representative levels of the vestibular nuclei are shown The rostro-caudal extent of the labelled cells approximated that of the abducens nucleus

Finally, a control animal (K-3-031273), where the horseradish peroxidase was injected into the middle ear revealed absence of labelled cells in the brainstem and cerebellum The




*List of abbreviations used in figures*

LVN = lateral vestibular nucleus.
MVN = medial vestibular nucleus.
SVN = superior vestibular nucleus.
DCN = dorsal cochlear nucleus.
VCN = ventral cochlear nucleus.
ASO = accessory superior olivary nucleus.
LSO = lateral superior olivary nucleus.
V = descending trigeminal root.
VI = abducens nucleus.
VII = facial nerve.
RB = restiform body.
VR = vestibular root.
LN = lateral (dentate) nucleus.
IN = interposed nucleus.
FN = fastigial nucleus.

*Fig. 2.* Outline drawings of the brain stem of K 2-031273 showing the location of labelled neurons (black dots) following injection of horseradish peroxidase into the right vestibule.

possibility of other efferent neurons being labelled with peroxidase was ruled out with this control.

Two groups of efferents that could conceivably be involved are 1) the motor nerves to the middle ear muscles and 2) autonomic preganglionic efferent neurons of the seventh and ninth cranial nerves. The stapedius and tensor tympani muscles are innervated by branches of the seventh and fifth cranial nerves. The nuclei of these nerves are located in the brainstem at the caudal and rostral levels of the vestibular nuclei. No labelled cells were seen in the facial nucleus or the motor trigeminal nucleus.

The autonomic efferents travel via (*a*) the pars intermedia of the seventh cranial nerve through the chorda tympani nerve to the submaxillary ganglion and (*b*) the tympanic branch of the ninth cranial nerve through the

tympanic plexus and lesser superficial petrosal nerve to the otic ganglion The cells of origin of these autonomic nerves are located in the superior and inferior salivatory nuclei respectively within the brainstem While their nerve terminals are far removed from the middle ear space, it was considered possible that the peroxidase could penetrate the intratympanic portions of the axons The absence of tracer in the brainstem of the control, however, provided evidence against this possibility

## DISCUSSION

While the phenomenon of anterograde axonal transport has been known for some time (Lasek, 1968, Ochs et al, 1967, Taylor & Weiss, 1965), the concept of retrograde transport of proteins from the nerve ending into the cell body has only been recently demonstrated (Kristensson et al, 1971, Kristensson & Olsson, 1971, Zacks & Saito, 1969) Several investigators have demonstrated that protein tracers such as Evans Blue albumin and horseradish peroxidase can be identified in motor neurons in the central nervous system after presentation of these protein tracers to their motor endplates This has been clearly demonstrated in the hypoglossal and vagus nerves as well as the motor nerves to limb muscles such as the gastrocnemius muscle These studies have been carried out in newborn animals because in the newborn animal the perineurium is open-ended peripherally (Zacks & Saito, 1969) as contrasted to adult animals where the perineurium constitutes a barrier for proteins to diffuse into endoneurium and contact the axon (Kristensson & Olsson, 1971) It is therefore possible to present an exogenous protein tracer such as horseradish peroxidase at the axon terminal, and then after a period of time, at least 10 hours and preferably 24 hours, the protein tracer can be identified in the cytoplasm of the neurons whose axons have been in contact with the protein tracer An important factor in determining the uptake of protein in the axons is the concentration of the tracer around the terminals This was substantiated in the present study where the greater concentration of peroxidase injected by the round window approach in K-2-031273 produced a stronger uptake and more cells labelled than in the animals injected via the oval window technique where the peroxidase leaked out of the vestibule The present study showed that, after injection of horseradish peroxidase into the vestibule, 5 out of 7 animals had consistent localization of horseradish peroxidase in small cells located just lateral to the abducens nucleus and ventro-medial to the ventral division of the lateral vestibular nucleus (Table I) While the amount of uptake in these cells via the oval window injection of the peroxidase was variable, this approach was technically easier and less likely to contaminate the cerebro-spinal fluid space On the other hand, while better concentration could be attained through a round window approach, in 2 of the 3 animals where this approach was used, the peroxidase entered the cerebro-spinal fluid space and was not useful for localization of the efferent neurons to the inner ear An indication that concentration of the horseradish peroxidase in the peri lymphatic space of the vestibule was important was clearly brought out by the amount of uptake of peroxidase in the labelled cells of animal K-2-031273 as well as the number of cells so labelled The increase in labelled [illegible] in this animal as compared to animal [illegible] 020273 lies primarily in the number of [illegible] tralateral efferent neurons that were [illegible] strated These neurons are located [illegible] distance from the side of injection [illegible] fore would require a greater [illegible] peroxidase to be labelled in the [illegible] period The total number of [illegible] neurons to one labyrinth as [illegible] study agrees with the numb[illegible] as revealed by degenerati[illegible] techniques This numb[illegible] estimated at 200–300 ([illegible])

The possibility that [illegible] brainstem lateral to [illegible]

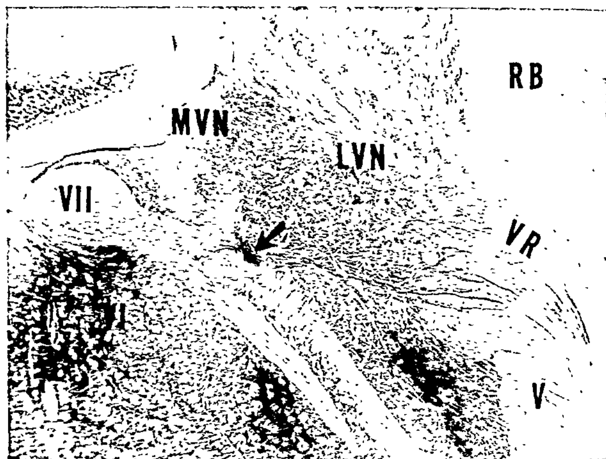



*Fig 3* Photomicrograph of normal adult cat brainstem demonstrating high acetylcholinesterase activity in a localized area ventro-medial to the lateral vestibular nucleus (*LVN*) and projecting fibers into the vestibular root (*VR*).

longed to autonomic portions of the seventh and ninth cranial nerves, or to the innervation of the stapedius or tensor tympani muscles was ruled out by the injection of the horseradish peroxidase into the middle ear of kitten K-3-032173 No uptake was seen in any cells of the brainstem of this animal

An additional indication that the labelled neurons are those of vestibular efferents is found in animal K-2-031273 (Fig 2) where some labelled cells were seen in the dorsal perimeter of the lateral superior olivary segment. This is precisely where the neurons giving rise to the uncrossed olivo-cochlear bundle have been located by other methods Undoubtedly some horseradish peroxidase travelled up the scala vestibuli and down into the upper portions of the scala tympani where diffusion through the osseous spiral lamina into the perilymphatic space of the organ of Corti provided the route for uptake of exogenous protein by efferent cochlear fibers. With a longer period of time and greater concentration of peroxidase in scala tympani more olivocochlear efferent neurons can be labelled with this technique (Warr, 1973)

The present location of efferent vestibular neurons is further supported by the acetylcholinesterase localization technique where an area of high activity has been noted medial and ventral to the lateral vestibular nucleus (Fig 3) Acetylcholinesterase positive fibers emanating from this location can be seen travelling into the dorsal portion of the vestibular

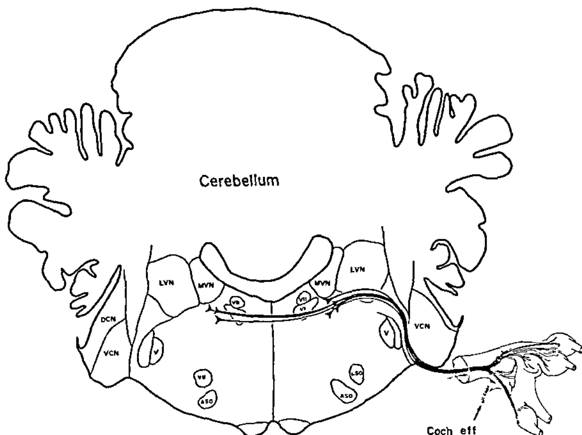



*Fig. 4.* Drawing of the origin, course and termination of the efferent vestibular pathway.

root where they become associated with the outgoing olivo-cochlear fibers. Furthermore, lesions producing axonal degeneration which have been used to demonstrate the peripheral portions of the bundle in the past have localized the most probable site of efferent vestibular neurons to an area near the ventral portion of the lateral vestibular nucleus or the reticular formation. Degeneration experiments such as these, however, have failed to indicate bilaterality to the origin of the vestibular efferents. Possible reasons for this failure to demonstrate a contralateral bundle are 1) difficulty in staining fine degenerating efferent fibers in the peripheral nerve segments with silver or myelin techniques and 2) the midline transections of the olivo-cochlear bundle were not deep enough to cut the axons of the contralateral efferent fibers. This would indicate that the axons of the contralateral vestibular efferent neurons cross the midline at a deeper level than olivo-cochlear fibers.

The drawing in figure 4 depicts the origin, course and termination of the vestibular efferent pathway and its relationships to the efferent cochlear bundle. The present location of the vestibular efferent neuron differs from the "nucleus interpositus" described by Rossi & Cortesina (1962). He described an entirely ipsilateral origin for the vestibular efferent system, one was located in the lateral vestibular nucleus giving rise to the "dorsal direct vestibular efferent bundle" and the second was the "nucleus interpositus" which was located

between the lateral vestibular nucleus and the descending trigeminal root and gave rise to the "ventral direct vestibular efferent bundle" Acceptance of this origin was not universal because of the previously mentioned difficulty in determining unequivocal retrograde changes in small neurons where scanty cytoplasm is normally seen and where eccentric nuclei may be present in the normal animal Carpenter (1960) on the basis of widespread retrograde cell changes following labyrinthectomy ascribed a bilateral origin in the cerebellar nuclei and several vestibular nuclei Again equivocal cell changes and the disproportionately large number of neurons involved, prevented acceptance of these studies as indicative of the origin of vestibular efferent fibers

A consideration of the location of the efferent neurons with regard to the incoming afferent innervation from the labyrinth is significant At their location, ventral to the medial and lateral vestibular nuclei, these neurons are located in an area where the descending branches of first order neurons from the cristae and maculae converge The opportunity is present, therefore, for direct termination of the afferent neurons from all vestibular sense organs onto the efferents This is not surprising when one considers that the axons of the efferent fibers travel out the vestibular nerve and disperse evenly to the hair cells in all of the sense organs

A number of physiological experiments on the efferent vestibular system have been conducted most notably those by Schmidt (1963), those of Gleisner & Henriksson (1964), and finally of Sala (1965) Of these, only that of Sala has attempted to stimulate the efferent vestibular system by placing the stimulating electrode within the vestibular complex His stimulating electrode was placed ventrally in the lateral vestibular nucleus (p 16 in Sala, 1965) and appears very near the location of the vestibular efferent neurons as described in this study The inhibitory effect on vestibular nerve activity was probably mediated via the contralateral efferent component In such a case, however, it may be noted that only half of the vestibular efferent influence to one labyrinth was being stimulated

With the precise location of the efferent vestibular neurons the opportunity now exists for more accurate physiological evaluation of this system A major reason for conducting such morphological studies is to aid in the design and interpretation of neurophysiological investigation

## ZUSAMMENFASSUNG

Die Lage der efferenten Neuronen im Stammhirn neugeborener Katzen wurde über den retrograden Transport von Proteinen mittels Meerrettich Peroxydase bestimmt Zwei bis drei Milligramm Meerrettich Peroxydase wurden bei 8 Katzen ins Vestibulum injiziert Nach 24 Stunden wurden die Tiere fixiert und ihre Stammhirne wurden geschnitten Die Farbung der Peroxydase zeigte die markierten Neuronen lateral auf beiden Seiten des Nucleus abducens an

## REFERENCES

Brodal A 1940 Modification of Gudden method for study of cerebral localization *Arch Neurol* (Chic) *43* 46

Carpenter M B 1960 Experimental anatomical physiological studies of the vestibular nerve and cerebellar connections In *Neural mechanisms of the auditory and vestibular systems* (ed G L Rasmussen & W R Windle) C C Thomas Springfield Ill

Gacek R R 1960 Efferent component of the vestibular nerve In *Neural mechanisms of the auditory and vestibular systems* (ed G L Rasmussen & W F Windle), pp 276–284 C C Thomas, Springfield Ill

— 1964 Efferent vestibular pathway In *The vestibular system and its diseases* (ed R J Wolfson) pp 99 116 U of Penn Press Phila, Pa

— 1967 Anatomical evidence for an efferent vestibular pathway *Third International Symposium on The Role of Vestibular System in Man s Exploration of Space* pp 203–213 Pensacola Fla

— 1969 The course and central termination of first order neurons supplying vestibular endorgans in the cat *Acta Otolaryng* (Stockh) Suppl *254*

Gacek, R R Nomura Y & Balogh K 1965 Acetylcholinesterase activity in the efferent fibers of the stato-acoustic nerve *Acta Otolaryng* (Stockh) *59* 541

Gleisner L & Henriksson N G 1964 Efferent and afferent activity pattern in the vestibular nerve of the frog *Acta Otolaryng* (Stockh) Suppl *192* 90

Graham, R C & Karnovksy, M J 1966 The early stages of absorption of injected horseradish peroxidase in the proximal tubules of mouse kidney ultrastructural correlates by a new technique *J Histochem Cytochem 14* 291

Kristensson, K & Olsson, Y 1971 The perineurium as a diffusion barrier to protein tracers *Acta Neuropath 17*, 127

Kristensson, K., Olsson, Y & Sjostrand, J 1971 Axonal uptake and retrograde transport of exogenous proteins in the hypoglossal nerve *Brain Res 32*, 399

Lasek, R 1968 Axoplasmic transport in cat dorsal root ganglion cells as studied with (³H)-L-leucine *Brain Res 7*, 360

Ochs, S, Johnson, J & Ng M H 1967 Protein incorporation and axoplasmic flow in motoneuron fibers following intra-cord injection of labelled leucine *J Neurochem 14*, 317

Rasmussen, G L & Gacek, R R 1958 Concerning the question of the efferent fiber component of the vestibular nerve of the cat *Anat Rec 130* 361

Rossi, G & Cortesina, G 1962. Il "sistema efferent colinergico cochleo vestibolare" *Minerva Otorinolaring 12*, 1

Sala O 1965 The efferent vestibular system Electrophysiological research *Acta Otolaryng* (Stockh), Suppl *197*

Schmidt, R S 1963 Frog labyrinthine efferent impulses *Acta Otolaryng* (Stockh) *56* 51

Sjostrand, J 1969 Rapid axoplasmic transport of labelled proteins in the vagus and hypoglossal nerves of the rabbit *Exp Brain Res 8*, 105

Smith, C A & Rasmussen G L 1967 Nerve endings in the macular and cristae of the chinchilla vestibule, with a special reference to the efferents *Third International Symposium on The Role of Vestibular System in Man s Exploration of Space*, pp *183–201* Pensacola, Fla

Taylor, A C & Weiss, P 1965 Demonstration of axonal flow by the movement of tritium labelled protein in mature optic nerve fibers *Proc Nat Acad Sci USA 54*, 1521

Warr, B 1973 Localization of olivo-cochlear neurons with horseradish peroxidase Presented at the Eighty sixth Meeting of the American Assoc of Anatomists, April 9, 1973, New York City

Zacks, S I & Saito A 1969 Uptake of exogenous horseradish peroxidase by coated vesicles in mouse neuromuscular junctions *J Histochem Cytochem 17*, 161

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# REINNERVATION AFTER RESECTION OF THE FACIAL NERVE

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*Abstract* After radical parotidectomy for carcinoma carried out in a five month-old child spontaneous return of the function of the facial musculature developed gradually during the second year after the intervention Electromyographic studies showed that reinnervation developed mainly from the homolateral (involved) side even though the contralateral (uninvolved) side evidently also participated in the reinnervation of the mouth sphincter Responses could be evoked from the superficial layers of the subcutis in the midface The authors therefore assume that in the case described the unexpected reinnervation was effected by dual innervation of the buccinator It is likely that in some persons this muscle is also activated by the motor fibres of the trigeminus

In recent years much interest has been paid to the physiology of the facial musculature due to the successful replacement of the missing segments of the facial nerve by nerve grafts and to the so far unresolved problem of the spontaneous return of the function of the facial muscles in patients whose seventh cranial nerve had been resected and no effort made to reconstruct its anatomical continuity by surgical intervention Spontaneous reinnervation was observed to occur more frequently after resection of the whole extratemporal portion than after loss of an endotemporal portion of the facial nerve

This unusual phenomenon is the subject of controversy and remains an unresolved problem The regenerating axons can reach the paralysed facial muscles in some uncommon manner, either via the homolateral or the contralateral seventh cranial nerve or via the fifth cranial nerve

To our knowledge only about 30 such cases have, so far, been reported in the literature Aberrant reinnervation was described for the first time by Martin & Helsper (1957 and 1960) though it had already been observed earlier by other authors Martin et al observed this phenomenon in eight cases in connection with total radical parotidectomy whereas Conley (1963) in five persons and Trajaborg & Siemssen (1972) in four patients in whom the same operation had been performed Other authors reported only singl cases of spontaneous reinnervation after los of the extratemporal portion of the sevent cranial nerve Silverstein et al (1967) encountered this phenomenon in one patien with a destroyed tympanic portion of th facial nerve by middle ear teratoma

The findings described in this paper might contribute to the problem of spontaneous reinnervation whose importance increases both from the theoretical and the clinical point of view

## CASE REPORT

A five month-old girl was referred to our clinic with an enlarging mass in the right parotid gland which had reached the size of a plum within a period of 3 months The tumour was

Presented on the occasion of the Congressus Oncologicus et Tumores Infantum held in Karlovy Vary on September 23rd 1972.



*Fig. 1.* Total facial nerve paralysis caused by a tumour.

hard and painful and led to a spontaneous development of total facial nerve paralysis (Fig. 1). Sialography disclosed destructive changes in the parotid gland. Fine needle aspiration biopsy established the malignant nature of the mass (Fig. 2). On May 29th 1970, total radical parotidectomy was performed. The sternocleidomastoid and masseter muscles and the cervical lymph nodes with the internal jugular vein were resected. The intervention was completed by tarsorrhaphy. Histopathology showed an immature cancer. The bioptic specimen from the lymph nodes was negative. Post-operatively, the right parotid region was irradiated with Cobalt$^{60}$ teletherapy (total dose 3 300 R).

The first voluntary motion of the mouth angle appeared 16 months after surgery. Supported by rehabilitation measures, the motion of the facial muscles gradually extended over the whole face. Two years post-operatively, the tumour did not recur and the face appeared symmetrical when at rest. The functional weakness was apparent, however, on emotional activity especially in the involved lower part of the face (Fig. 3).

The electromyographic (EMG) studies of the buccinator revealed a simple or intermediate response during voluntary motion (Fig. 4 *a*). The reaction of the orbicularis oris muscle was recorded on stimulation with a bipolar surface electrode. The latency time measured on stimulation of the facial nerve on the affected side was 5.8 msec (Fig. 4 *b*) whereas that from the normal side was 10 msec (Fig. 4 *c*). The response from the depressor oris inferior muscle had a latency time of 5 msec, and from the zygomatic muscle it did not appear at all. No response was obtained on stimulation of the plexus cervicalis. When the bipolar needle stimulation electrode was inserted superficially in mid face, the response was recorded from the orbicularis oris muscle. On deeper insertion in front of the external auditory canal, and toward the foramen stylomastoideum, no response could be obtained. The evaluation of the results was difficult because the examination was painful and the child restless.

## DISCUSSION

The spontaneous and unexpected return of motion following surgical section or resection at a distance of some centimetres distal to the ganglion geniculi is made possibly only by motor fibres belonging either to the seventh or the fifth cranial nerve. The voluntary motor impulses cannot be transmitted to the paretic muscles via sensory or vegetative nerves (Wright, 1967). The axoplasmatic flow from the central stump of the facial nerve disappears in the scarring area. It is unlikely that outgrowth sprouts of the main nerve trunk could persist in such a milieu and penetrate up to the paralysed facial muscles.

Our interpretation of the spontaneous reinnervation is based on the above mentioned

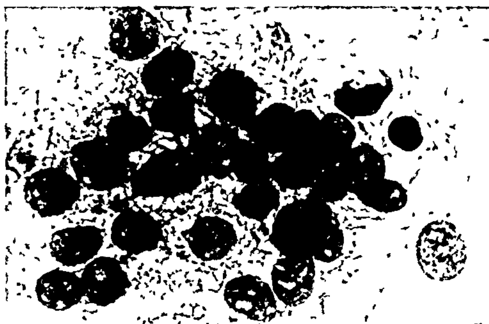


*Fig 2* Smear of the parotid gland tumour (Pappenheim ME) × 1 200 Group of tumour cells with veiled grey bluish plasma Anisomorphy anisochromy and pathological nucleols

grounds The central communications between the motor nuclei of the facial and trigeminal nerve are well known (Braus 1960) The paretic facial musculature can receive the impulses via three possible anatomical pathways through the motor nucleus of the facial nerve from the cortex

1) *Contralateral reinnervation from the facial nerve of the uninvolved side*

It is well known to the neurologist that the unaffected side can partly substitute the function of the involved side The mandibular branches of the facial nerve on both sides have overlapping areas of innervation This observation was made by us while carrying out a dissection study of the peripheral division of the extratemporal portion of the seventh cranial nerve in several persons (Černy et al 1970) The contralateral reinnervation from the non paretic side brings about only the motion around the mouth angle This phenomenon might not always be conditioned by overgrowth of the axons inside the sphincter oris muscle According to present knowledge, the maximum participation of the uninvolved side in the anomalous innervation is 20% (Passerini 1968) A more important role is ascribed to contralateral reinnervation by Fisch (1968)

This can be verified either by procaine infiltration at the circumference of the mouth angle on the uninvolved side or by comparison of the latency time after simultaneous stim



*Fig 3* Child 2 years after operation

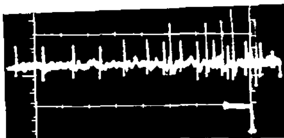


*Fig 4* (*a*) Contraction activity during crying from the m buccinatorius dx Potentials of two motor units with frequency of 20–40 cycles/sec Calibration 100 $\mu$V/50 msec

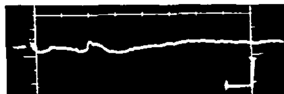


*Fig 4* (*b*) Evoked potential from m orbicularis oris dx Stimulation near the tragus of the contralateral side with bipolar surface electrode Calibration 150 $\mu$V/5 msec

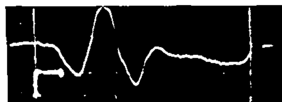


*Fig 4* (*c*) Evoked potential from m orbicularis oris dx Stimulation near the tragus on the ipsilateral side with bipolar surface electrode Calibration 150 $\mu$v/2 msec

ulation from the paralytic and the normal unaffected side during EMG studies For ethical reasons, we did not use nerve bloc with anesthetics but the electromyographic stimulation tests Thus proof could be provided that the mouth sphincter was partly innervated from the contralateral side

*Reinnervation from the facial nerve of the affected side*

A dominant role in the spontaneous return of the function of the paretic facial muscles is attributed to the involved seventh cranial nerve by Conley (1963) Unlike other investigators, he did not succeed in inhibiting all the renewed functions of the facial muscles by anesthesia of the maxillary and mandibular branches of the fifth nerve The results obtained showed a rarefaction in muscle activity on potentials at voluntary activity, which occurs in neurogenic atrophy or at reinnervation According to Conley, the phenomenon of spontaneous reinnervation might be due to unknown aberrant connections via the branches of the facial nerve He also takes into consideration the geniculate ganglion with its two petrosal nerves and the extracranial ganglia which develop from the seventh cranial nerve (Gasser, 1967) He concludes that under abnormal conditions the impulses might reach the paretic muscles along these hardly imaginative anatomical pathways

(*a*) The ganglion oticum, the junction of the fibres of the fifth and seventh cranial nerve, may represent an important point of homolateral spontaneous reinnervation of the mimetic muscles The motor fibres of the facial nerve reach the ganglion oticum either via the lesser petrosal nerve through a strong communication cum plexu tympanico (Villiger, 1943), or via the communicating ramus between the lesser and greater petrosal nerve on the upper pyramidal surface (Sobotta & Figge, 1963)

(*b*) The composition of the neural elements of the ganglion sphenopalatinum and of the greater petrosal nerve is not in accord with the requirements of aberrant innervation This nerve contains only rarely insignificant amounts of motor fibres (Gardner et al, 1963)

(*c*) The severed central stump of the main trunk of the seventh cranial nerve might also serve as a source of homolateral reinnervation Binns (1972) expressed this viewpoint on the basis of the results obtained from experiments carried out on cats McCoy & Boyle (1971) are of the same opinion This hypothesis is, however, hardly acceptable as far as man is concerned because it contradicts the

theoretical knowledge and the clinical experiences derived in neurosurgery

The better innervation from the affected side was demonstrated in our patient It is difficult, however, to determine whether the restored function of the facial muscles is attributable to the seventh cranial nerve

*Reinnervation from the fifth cranial nerve*

In aberrant innervation, only the motor branches of the portio minor of the trigeminal nerve may be of use This portion innervating the masticatory muscles is characterized by a variable ramification and its branches by a variable course Silverstein et al (1967) and Mundnich (1967) assume on the basis of clinical estimations that anastomoses between the motor component of the fifth and the seventh cranial nerve exist in ca 5–15% of all humans However, a reliable proof in that respect could not be provided as yet These rami communicantes probably arise during embryonal life, since the trigeminal and facial nerves are related, as far as the development is concerned, to the same branchial origin (Gasser, 1967)

Thus many authors have motivated the observed spontaneous reinnervation They argue that the assumed loss of aberrant innervation, which has been restored after previously carried out surgical intervention, does not always take place, when extended radical reoperation for recurrent parotid cancer is performed After the local nerve block of the maxillary and mandibular branches of the fifth cranial nerve or after alcohol injections, indicated in cases of accidentally coinciding trigeminal neuralgia, only a transient cessation of face motion is noticed A spontaneous reinnervation may be effected by the following pathways of the trigeminal nerve

(*a*) Via the masseteric nerve which supplies both the similarly named muscle and the deep layer of the temporal muscle whilst the superficial layer is innervated from the facial nerve

(*b*) Via a thick anastomosis between the buccal branch of the fifth nerve and the buccal branch of the seventh cranial nerve (Braus, 1960) This sensory branch penetrating the buccinator is joined by a small bundle of motor fibres (Hill, 1946) which also seem to contribute to its innervation

(*c*) Via the large communicating rami between the auriculo-temporal and facial nerves The presence of these anastomoses, which are 1–3 in number, and the connections with the otic ganglion have recently been reported by Baumel et al (1971) They contain, besides the secretomotor twigs and vasodilatator fibres destined for the glandular vessels also motor fibres for myoepithelial cells and smooth muscles of the duct system of the parotid gland

## CONCLUSION

In our patient, spontaneous reinnervation developed from the ipsilateral side We could not establish how and along which anatomical pathway it was effected, whether the anastomoses lying inside or around the mentioned muscles influenced it or whether the outgrowth of axons from the masticatory muscles penetrated into the denervated mimetic muscles The evoked potentials were recorded from the superficial layers of the subcutis in the middle of the face where the fibres of the buccal branches of the fifth and seventh cranial nerves are situated, both of them lying above the buccinator

We assume that in some persons this muscle is also supplied from the trigeminal nerve Dual innervation of the musculus buccinatorius or connections between motor fibres from buccal branches of both cranial nerves may play an important role in spontaneous reinnervation of the facial muscles

Obviously, the boundary-line of the development and the innervation of the mimetic and masticatory muscles is not so sharp as described in textbooks on embryology and in anatomical atlases The authors are of the opinion that, besides all the other pathways, the motor portion of the trigeminal nerve

might play its role in spontaneous reinnervation Further details and specific studies concerning the variability of innervation of this region which arises in prenatal life, are still required

## ACKNOWLEDGMENTS

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## ZUSAMMENFASSUNG

Nach radikaler Parotidektomie wegen Carcinoma die bei einem 5 Monate alten Saugling durchgefuhrt wurde kam es im Laufe des zweiten Jahres nach dem Eingriff zu spontaner Reinnervation der Gesichtsmuskulatur Elektromyographisch wurde festgestellt dass die Innervation des Mundsphinkters vorwiegend von der homolateralen (betroffenen) Seite ausging obwohl sich daran auch die kontralaterale (gesunde) Seite beteiligte Es ist uns gelungen aus den Oberflachenschichten der Unterhaut im mittleren Gesichtsfeld in der Region des Buccinators Potentiale hervorzurufen Die Autoren schliessen daraus dass im gegebenen Fall die unerwartete Funktionsruckkehr durch zweierlei Innervation dieses Muskels zustande kam Sie nehmen an dass dieser Muskel bei manchen *Personen auch durch die motorischen Nervenfasern* des Trigeminus versorgt wird

## REFERENCES

Baumel J J Vanderheiden J P & McElenney J E 1971 The auriculotemporal nerve of man *Amer J Anat 130* 431
Binns P M 1972 Facial nerve homografts *Arch Otolaryng* (Chic ) *95* 342
Braus H 1960 *Anatomie des Menschen* 2nd ed vol 3 J Springer Verlag Berlin–Heidelberg–Gottingen
Černý L Obručníkova J Kranichova H & Lisoněk P 1970 Beitrag zur chirurgischen Anatomie der Verzweigung des Nervus facialis im Gesicht *Acta Univ Palackianae Olomucensis 55* 77
Conley J 1963 Spontaneous return and facial nerve grafting *Arch Otolaryng* (Chic ) *77* 643
Fisch U 1968 Zur Frage der Reinnervation der Gesichtsmuskulatur nach Resektion des zugehorigen Nervus facialis *Pract Otorhinolaryng* (Basel) *30* 377
Gardner E, Gray D H & O Rahilly R 1963 *Anatomy* 2nd Ed W B Saunders Co Philadelphia–London
Gasser R F 1967 The development of the facial muscles in man *Amer J Anat 120* 357
— 1967 The development of the facial nerve in man *Ann Otol 76* 37
Hill R T 1946 *Anatomy of the head and neck* H Kimpton London
Martin H & Helsper J 1957 Spontaneous return of the function of the N VII following surgical section *Ann Surg 146* 715
— 1960 Supplementary report on spontaneous return following surgical section or excision of the seventh cranial nerve in the surgery of parotid tumours *Ann Surg 151* 538
McCoy E G & Boyle W F 1971 Reinnervation of the facial muscles following extratemporal facial nerve resection *Laryngoscope 81* 1
Mundnich K 1967 Zur Problematik und zu einigen schwierigen Situationen der reparativen Facialischirurgie *Chir Plast Reconstr 3* 86
Passerini D 1968 Contralateral reinnervation in facial palsy *Electromyography 8* 115
Silverstein H Griffin W L & Balogh K 1967 Teratoma of the middle ear and mastoid process A case with aberrant innervation of the facial musculature *Arch Otolaryng* (Chic ) *85* 243
Sobotta J & Figge F H 1963 *Atlas of human anatomy* Hafner Publ Co New York
Trajaborg W & Siemssen S O 1972 Reinnervation after resection of the facial nerve *Arch Neurol* (Chic ) *26* 17
Villiger E 1943 *Die Periphere Innervation* 8th ed, Verlag W Engelmann Leipzig
Wright S 1967 Applied physiology *SZdN Prague*

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# COMPRESSION AND ISCHAEMIA OF THE FACIAL NERVE

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*Abstract* Two factors possibly involved in ischaemic facial paralysis in man were investigated in the cat *compression* by injecting fluid between the nerve fibres and *ischaemia* due to circulatory arrest The nervous function was assessed by observation of nerve action potentials, the lesions by histological examinations About $3^1/_2$ hours of suprasystolic compression was critical The effect is attributed to occlusion of the arterial circulation Infrasystolic compression had less influence, infradiastolic compression no influence Histological examinations demonstrated disruptions of the nerve fluid between the nerve fibres and vascular stasis An increased vulnerability of the nerve to ischaemia is attributed to the presence of oedema between the nerve fibres If the theory about ischaemic paralysis in man is correct, a treatment interrupting the vicious circle of compression and ischaemia should be instituted in severe cases within the first day(s) in order to prevent denervation

The prevailing theory about the mechanism of acute idiopathic peripheral facial paralysis (Bell s palsy, rheumatic facial paralysis, facial *paralysis a frigore) is that of combined primary* and secondary ischaemia This theory, advanced by Kettel (1947), Hilger (1949), Sullivan & Smith (1950), is inspired *inter alia* by the experimental work of Denny Brown & Brenner (1944 *a b*) It can be summarized as follows (Fig 1) a local arteriolar constriction (the primary ischaemia) provokes an increase in permeability of the arteriolar wall due to hypoxaemia, the ensuing transsudate then compresses the facial nerve fibres, an impairment of the venous and lymphatic return of the circulation causes more oedema compressing the nerve fibres, and ischaemia of secondary nature A vicious circle is created in this way Theoretically, the nerve fibres could be saved by any therapeutic action which breaks the vicious circle before they have degenerated Unfortunately, the cause of the arteriolar constriction is not known, it may be allergic, toxic, endocrine or psychic (Miehlke, 1960) An unequivocal explanation may never be found, since various factors are capable of triggering the same pathogenetic mechanism (Zulch, 1970) The term "ischaemic paralysis" for this condition was introduced by Kettel (1954) Since the ischaemia is generally accepted as the triggering factor in this paralysis (Miehlke, 1960) that name will be used here

Our study was limited to the vertical (mastoid) part of the facial nerve which is in our opinion the most vulnerable part of the nerve *for several reasons In the human facial* canal it is the only segment where the nerve fibres are collected into a single fascicle (Sunderland & Cossar, 1953) and, at the same time are surrounded by a thick perineurium of 25 to 35 $\mu$ formed by concentric layers of dense collagenous connective tissue with few elastic fibres (James, 1961) Nerve fibres in monofascicular nerves are more susceptible to compression than fibres in multifascicular ones (Sunderland, 1968), and the hyperkalaemic depolarization of the nerve fibres by ischaemia may be additive in closely packed adjacent nerve fibres (Fox & Kenmore, 1967) Moreover, in a retrospective study of about 300 de

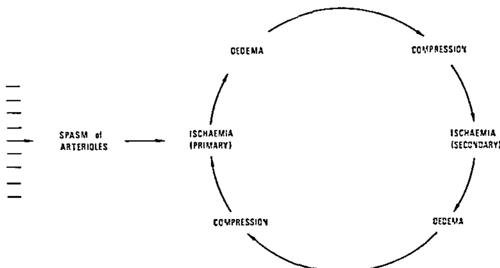



*Fig 1* Schematic representation of the pathogenesis in ischaemic facial paralysis

compressions of the vertical part of the nerve in cases of ischaemic paralysis we have seen not a single case of recurrent paralysis of a nerve that had been operated upon On the contrary, among 440 patients with ischaemic paralysis 19 non operated patients had a second attack of facial paralysis, 12 of them within 10 years after the first attack, and 2 non-operated patients even had 3 attacks at the same side of the face within 26 years (Devriese & Pelz, 1969) On the other hand we have seen a second attack of ischaemic paralysis after decompression of the nerve of the other side in a few instances Since decompression of the vertical part seems to exclude a recurrence on the same side, one may surmise that the pathology is localized chiefly in that segment of the nerve

Cats were chosen for our experiments since in the vertical part of the facial canal their facial nerve is also monofascicular and surrounded by a thick perineurium Two factors involved in the ischaemic theory, viz. the *compression* by oedema and the *ischaemia* were studied inside the facial canal For a more extensive description of the experiments the reader is referred to an earlier publication (Devriese, 1972 *b*)

## METHODS

The right zygomatico-orbital branch of the facial nerve of the cat was stimulated antidromically by a constant-current stimulus with a bipolar electrode The monophasic nerve action potentials were recorded by means of a glass electrode from the facial nerve that was cut in the middle ear just distal to the geniculate ganglion The *compression* of the nerve fibres was realized by injecting Locke's solution for cats (Eyzaguirre, 1960) into the facial nerve within the sheath in the vertical segment of the facial canal This method, originally used by Jain & Sharma (1964) in the rabbit, was modified substantially The pressure system for "infusion compression" is depicted in Fig 2 The 25 gauge hypodermic needle was occluded at the tip and polished in such a way as to locate the tip more centrally Small holes of 0 3 mm were drilled in the lateral walls as near as possible to the tip Any sharp edge was carefully polished away The pressure system was filled with Locke's solution corresponding to the extracellular fluid Heparin (0 6‰) was added to prevent clogging of the needle The hypodermic needle was inserted under the

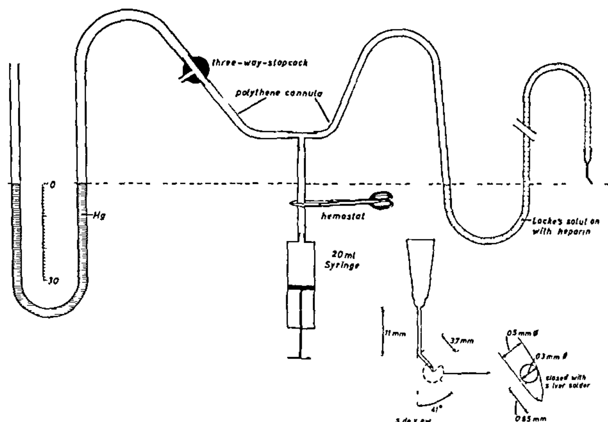



*Fig 2* Pressure system for infusion compression of the facial nerve In the right lower corner a schematic description of the modified hypodermic needle is given

operating microscope (magnification 10×) via the stylomastoid foramen into the facial nerve This was done under continuous inspection of the action potential on the oscilloscope The needle was fixed on a micromanipulator, the mercury manometer was adjusted to the same level as both the needle and the facial nerve

The blood pressure of the cat was measured by means of a catheter introduced into the abdominal aorta In 12 cats the blood pressure values ranged 130–160 mmHg/systolic and 90–120 mmHg/diastolic

In suprasystolic compression the pressure inside the compression system was kept between 150 and 320 mmHg, in infrastolic compression below the systolic blood pressure but above the diastolic blood pressure, and in infradiastolic compression under the diastolic blood pressure

The facial nerve with the needle inside it was prepared for histological investigation The fixation was initiated in vivo with the pressure still on the nerve by dripping SUSA fixative (Romeis, 1968) on the stylomastoid foramen for about 15 min After decapitation of the animal the temporal bone was removed for further fixation in SUSA fixative, dehydration in an alcohol series, embedding in Rollwax 1 (Lamb, London) and decalcification in 5% nitric acid in a saturated solution of picric acid (70% ethanol)

*Ischaemia* was obtained by arrest of the blood circulation at the end of an experiment A saturated solution of potassium chloride (3 mol) was injected intravenously

## RESULTS

### 1 *Suprasystolic compression*

*Electrophysiology* Fig 3 gives a three-dimensional display of the compound nerve action

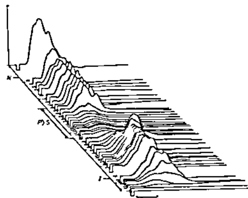


*Fig 3* Three-dimensional display of nerve action potentials before during and after 105 min of reversible suprasystolic infusion compression The first curve of the experiment is displayed in the left upper corner the last one in the right lower Each curve represents 500 averaged action potentials Calibrations 250 μV (vertical bar) and 1 msec (horizontal bar) The time (oblique axis) is indicated in hours *N* indicates the insertion of the needle *P>S* suprasystolic compression *I*, ischemia Experiment 41 SD (stimulus duration) 150 μsec SI (stimulus intensity) 300 μA MA (maximal amplitude in the experiment) 189 μV, RT (total recording time of the experiment) 7 h 27 min

potentials during the experiment The nerve was compressed for 105 min The compression was followed by an almost complete disappearance of nerve activity As soon as the pressure was released the action potential reappeared Finally, the action potential became about the same as before the compression although small differences in recovery time in certain fibres groups were still evident 2 hours after compression This experiment ended with recording of action potentials after arrest of the circulation (ischaemia) Fig 4 gives a survey of the action potential under the influence of compression of 210 minutes' duration After release of pressure, the amplitude of the nerve action potential remained negligible during the remainder of the observation period

Table I and Fig 5 give a survey of 9 experiments during which infusion compression of increasing duration was performed The amplitude of the action potential in percent of the amplitude before infusion compression (*ordinate*) is plotted against the time from the onset of compression (*abscissa*) After release of the pressure the amplitudes regained their original values in 6 of the experiments Generally, the longer the compression lasted, the slower was the return of nervous activity Although the amplitude of the action potential recovered in experiment 48 after 180 min of compression there was a change in shape and a delay of the action potential In experiments 60, 62 and 67 where the compression lasted for 210 or 245 min a rapid return of action potentials could no longer be observed There is a distinct difference in the effect of release after short and after long periods of compression Clogging of the needle was excluded after removal of the needle at the end of the experiment

*Histology* In Plate 1 the site in the nerve where the tip of the needle was located is shown Around the insertion site the nerve fibres are packed together In other parts of the nerve, oedema and disruption can be seen At a higher magnification (Plate 2), in a section likewise treated with the Mallory Azan connective tissue technique, the dilatations of

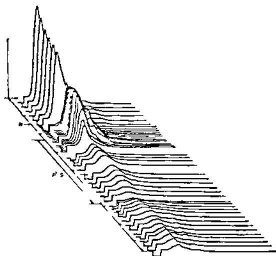


*Fig 4* Two-hundred and ten minutes of suprasystolic infusion compression Expt 60 SD 280 μsec SI 600 μA MA 374 μV RT 8 h 25 min The nerve action potential remained negligible after relief of the pressure

the endoneural connective tissue between the nerve fibres and vascular stasis can be observed

*2 Infrasystolic, infradiastolic, and mixed compressions*

An example is given in Fig 6 The blood pressure of the cat, the pressure in the infusion compression system, and the parameters of the action potential during the same experiment are given in Fig 7 At the beginning of the experiment, infradiastolic compression of 70 mmHg for 50 min did not influence the action potential Infrasystolic compression of 125 mmHg was then attempted for 3 hours After 75 min, however, the systolic blood pressure of the cat decreased and the compression exceeded the systolic blood pressure The amplitude reacted immediately Fifty five minutes later the blood pressure increased again and the effect of compression decreased After release of pressure the action potential returned to the precompressional level Finally, the nerve was submitted to 42 min of suprasystolic compression and to ischaemia

*3 Ischaemia*

Ischaemia was studied in 12 cats in 2 of the control group, in 1 after a decompression operation and in 9 after a reversible pressure block of the nerve (the nerve block was judged reversible when the action potential recovered in amplitude and configuration after release of the pressure) In Fig 8 the maximal amplitude of the action potential (in $\mu$V) is plotted against the time after arrest of the blood circulation (in minutes)

The ischaemia in experiments 31 and 89 was preceded by a control experiment in experiment 26 by a successful decompression of the nerve, and in the other 9 by a reversible compression It is seen from the figure that the influence of ischaemia in experiments 26, 31 and 89 differed distinctly from that in the other nine In the former case the nerve resisted the process of ischaemia for a relatively

Table I *Suprasystolic infusion compression of the facial nerve for more than 40 minutes in 9 cats* See also Fig 5

| Exp. no | Action potential after insertion of needle (percentage of initial value) | Amplitude before applying pressure ($\mu$V) | Duration of pressure (minutes) |
|---|---|---|---|
| 72 | 44 | 96 | 42 |
| 38 | 64 | 170 | 58 |
| 55 | 32 | 100 | 76 |
| 41 | 50 | 108 | 105 |
| 36 | 50 | 219 | 122 |
| 48 | 90 | 373 | 180 |
| 62 | 67 | 604 | 210 |
| 60 | 70 | 190 | 210 |
| 67 | 100 | 516 | 245 |

long time, the action potential decreased to 50% after 17 to 45 min, then it broke down rapidly In the latter case the action potential decreased more rapidly after the onset of ischaemia, falling within 15 min to 50%, thereafter it fell more slowly

## DISCUSSION

The difference between the normal action potential and that observed after a pressure block must be due either to inactivity of nerve fibres or to slower conduction in the depressed area or to both (Gasser & Erlanger, 1929) Due to the short distance of conduction the nerve fibres groups could not be differentiated in the action potential The fact that the action potential never disappeared completely during compression could be attributed to action potentials generated in the intact part of the nerve fibres distal to the nerve block The function of the nerve can thus be influenced by injecting fluid into it It should further be stressed that this was done in the normal anatomical situation of the facial nerve inside the facial canal

As demonstrated in experiment 72 (Figs 6 and 7) the nerve action potential reacted sharply to small variations in blood pressure around the systolic blood pressure This proves that the actual pressure inside the

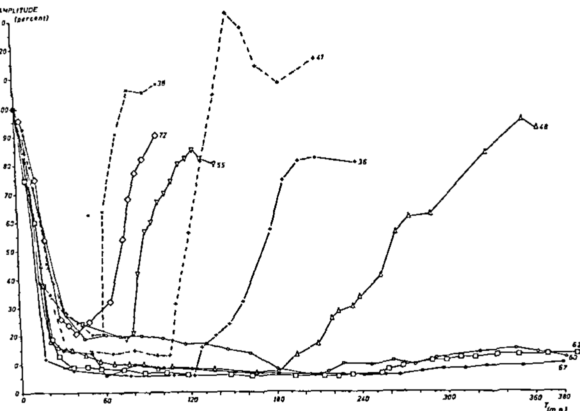


*Fig 5* Amplitudes of nerve action potentials during and after suprasystolic infusion compression of the facial nerve *Ordinate* amplitude in percent of the amplitude before compression *Abscissa* time in minutes from the onset of compression The figure at the end of each curve indicates the number of the experiment See also Table I

nerve corresponded to the pressure in the infusion compression system and that the effect of infusion compression is clearly related to the blood pressure of the animal Since the most distinct effects were seen during suprasystolic compression, the conclusion is reached that the arterial circulation of the nerve must play a determining part The initial changes caused by localized pressure are probably due to the associated ischaemia even if subsequent more pronounced structural changes are the results of anatomical deformation by the compression (Sunderland, 1968)

About 3 1/2 hours of suprasystolic compression appeared to be the critical duration of compression compatible with early functional recovery Infrasystolic compression above the diastolic blood pressure had little influence on the nerve action potential, as soon as it exceeded the systolic blood pressure the influence of suprasystolic compression became distinct (Fig 7) The effect of infrasystolic compression may be provoked by occlusion of the venous return with preservation of the arterial supply

Infradiastolic compression did not influence the nerve function In our opinion this is due to persistence of the circulation

The action potential decreased faster after ischaemia than after compression This can be explained by the fact that infusion compression required about 40 min to build up a nerve block by accumulation of a sufficient amount of fluid between the nerve fibres It should also be noted that the pressure block

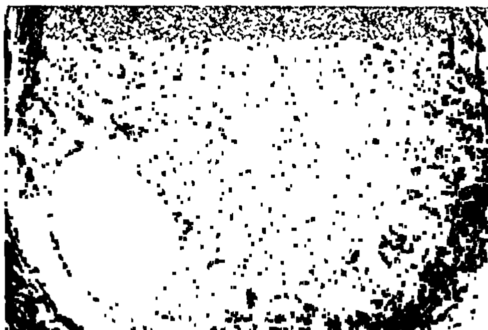


*Plate 1* Transverse action of the facial nerve submitted to infusion compression for one hour Around the site where the tip of the needle was localized (*left*) the nerve fibres are packed together Dilatations of the endoneural space are shown in the other parts of the nerve Cat 84 Mallory–Azan × 180

was limited to a small part of the facial nerve in the facial canal, whereas ischaemia involved the total length of the nerve under study In vivo, compression cannot be realized without interference with the circulation in the nerve The results clearly indicate a difference between the influence of ischaemia on intact nerves and on nerves previously subjected to infusion compression The only difference between the two groups of nerves was that in the second group Locke's solution with heparin was injected into the nerve Although the nerve action potential recovered after the short period of compression these nerves were more vulnerable to ischaemia This could be explained by the fact that fluid between the nerve fibres prevented diffusion of oxygen from the surrounding tissue towards the nerve

It follows that if oedema is indeed present between the nerve fibres in cases of ischaemic paralysis, the present experimental evidence demonstrates the importance of maintaining an adequate blood supply to the nerve during its surgical decompression (Miehlke, 1960, Kettel & Jongkees, 1963)

It has been demonstrated experimentally that decompression of the vertical part of the normal facial nerve can be performed without measurable damage to nerve function if performed with the utmost care (Devriese, 1972 *a*) Interference with the circulation of an oedematous nerve is probably more hazardous

Although the hypothesis of combined primary and secondary ischaemia in ischaemic paralysis in man is accepted, several factors still have to be proven The swollen appearance of the nerve after slitting the nerve sheath has been regarded as evidence of pathological swelling under pressure but may occur also when the perineurium of a normal nerve is opened (Sunderland & Cossar, 1953)

According to Jongkees (1965), however, the abrupt ending of the bulging of the decompressed oedematous nerve, in contrast to the non-bulging part, is the typical feature of ischaemic paralysis The localization of the

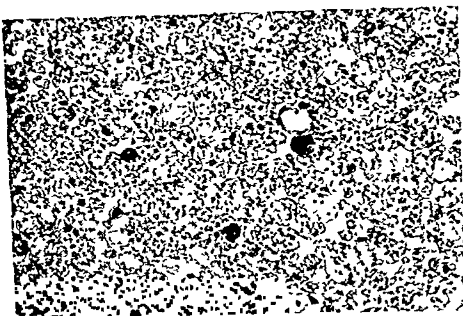


*Plate 2* Higher magnification of a facial nerve submitted to compression for 1 hour The endoneural space is dilated Note the filling of small venules and capillaries (stasis) and the dilated arteriole Cat 79 Mallory–Azan ×500

swelling may also vary, it may involve the tympanic portion of the nerve (Williams, 1959) or even the part of the facial nerve proximal to the geniculate ganglion (Fisch & Esslen, 1970) Histological examinations of the facial nerve or the chorda tympani in cases of ischaemic paralysis have been reported by Jongkees (1954), Kettel (1959), Miehlke (1960), McGovern (1970), and others Degenerative signs in the myelin sheaths (swelling) and less frequently in the axons, were found No apparent signs of inflammation such as infiltration by leucocytes were reported Examinations of the epineurium of the chorda tympani (Jongkees, 1954, Sade et al, 1965, Sade, 1972) was negative no oedema, no inflammatory changes or blood vessel anomalies could be discerned The oedema, however, could have disappeared before histological examination was performed (Kettel, 1947) No definite conclusion about the pathology of ischaemic paralysis can be reached without studies of normal temporal bones (Anson et al, 1970), more autopsy studies using advanced histological techniques (Fowler, 1963), and careful examination of the patients to either diagnose or exclude other diseases Finally it should be noted that only very few attempts have been made to measure the pressure inside the nerve (Jongkees, 1968), these allowing no definite conclusion

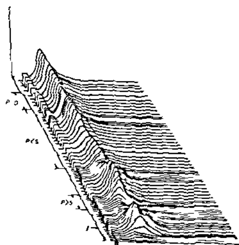


*Fig 6* Three periods of compression of the facial nerve Infradiastolic compression ($P < D$) for 50 min infrasystolic ($P < S$) for 180 min and suprasystolic ($P > S$) for 42 min *I* Ischaemia Exp 72 SD 140 μsec SI 600 μA MA 97 μV RT [illegible]

If the theory of combined primary and sec-

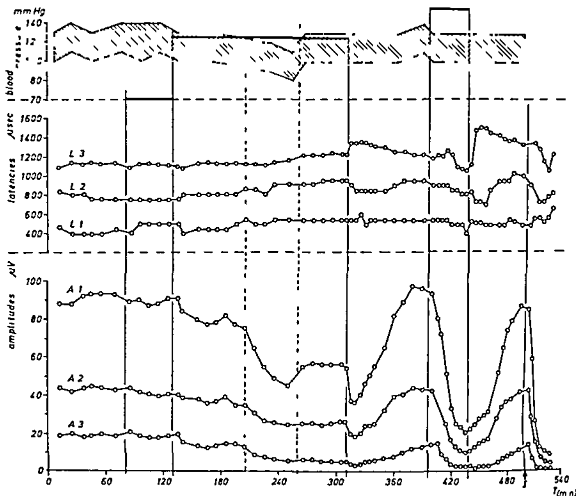



*Fig 7* Infradiastolic infrasystolic and suprasystolic infusion compression of the facial nerve Same experiment as in Fig 6 Six parameters of the nerve action potential are also represented L3 latency time to the centre of gravity L2 latency time to the maximum of the derivative L1 latency time to the onset A1 maximum amplitude A2 approximate surface A3 amplitude of the derivative

ondary ischaemia starting the vicious circle of ischaemia and compression is correct, the sudden ischaemic paralysis in man can be explained by occlusion of the arterial circulation to the nerve In the cat a critical duration of nerve block was reached after about $3\,^1/_2$ hours of interruption of the arterial circulation Under the influence of infrasystolic (supradiastolic) compression the nerve function decreased more slowly and would eventually have disappeared completely Unfortunately, this could not be checked since the experiments could not be continued for more than about 400 minutes

Our results support the statement of Bunnell (1952) that the only possible time for prophylaxis or prevention of denervation is during the first three hours if the blockade of the circulation is complete If it is partial it would presumably take a few days longer Naumann et al (1968) also suggested on clinical grounds that the most critical period for the nerve is the first 24 hours The best results of treatment can then be reached by interrupting the vicious circle of compression and ischaemia in severe cases at the earliest possible moment the first day or days This could explain the results recently reported after early treatment with corticosteroids (Taverner et al , 1971, Adour et al , 1972)

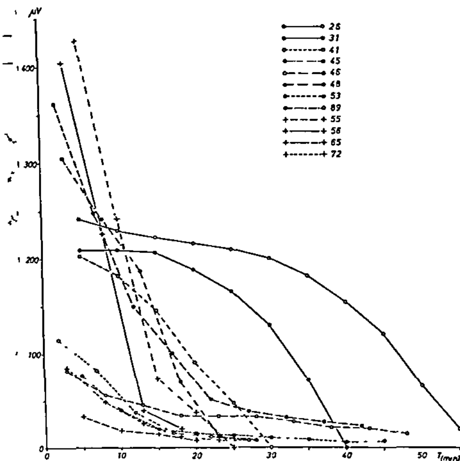



Fig 8 Influence of ischaemia on the maximal nerve action potential in 12 cats The maximal amplitude of the action potential (in µV) is plotted against the duration of ischaemia (in minutes) *Inset* the number of the experiment

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## ZUSAMMENFASSUNG

Zwei Faktoren welche moglicherweise auf die ischamische (Bellsche) Fazialislahmung beim Menschen bezogen werden können, wurden bei der Katze untersucht *Kompression durch Einspritzung von Flüssigkeit* zwischen die Nervenfasern, und *Ischämie* durch Blockierung der Blutzirkulation. Die Nervenfunktion wurde mittels Beobachtung der Nervenaktionspotentiale geschatzt, und die Lasionen wurden histologisch untersucht Ungefahr 3 ¹/₂ Stunden suprasystolische Kompression waren kritisch, was der Abschliessung der arteriellen Zirkulation zuzuschreiben ist. Infrasystolische Kompression hatte weniger und infradiastolische Kompression keinen Einfluss. Bei histologischen Untersuchungen wurden Einrisse im Nerv, Flussigkeit zwischen den Nervenfasern und vaskulare Stauungen gefunden Ödem zwischen den Nervenfasern verursachte eine grössere Anfalligkeit der Nervenfasern fur Ischamie Ist die Lahmungstheorie durch Ischamie beim Menschen korrekt, so musste man versuchen, den Circulus vitiosus von Kompression und Ischamie innerhalb der ersten Tagen durch zu brechen, um Denervation vorzubeugen

## REFERENCES


Adour K A Wingerd J Bell D N Manning J J & Hearly J P 1972 Prednisone treatment for idiopathic facial paralysis (Bells Palsy) *New Engl J Med 287* 1268

Anson B J Donaldson J A Warpecha R L & Rensink M J 1970 The facial nerve sheath and blood supply in relation to the surgery of decompression *Ann Otol 79* 710

Bunnell S 1952 Summation of papers on management of facial paralysis *Arch Otolaryng* (Chic) *55* 417

Denny Brown D & Brenner C 1944*a* Paralysis of nerve induced by direct pressure and by tourniquet *Arch Neurol Psych 51* 1

— 1944*b* Lesion in peripheral nerve resulting from compression by spring clip *Arch Neurol Psych 5?* 1

Devriese P P 1972*a* Experimental facial nerve decompression in the cat *Arch Otolaryng* (Chic) *95* 350

— 1972*b* Experiments on the facial nerve A study of nerve action potentials in the cat *Verh Ned Akad Wet 61* 1

Devriese P P & Pelz P G 1969 Recurrent and alternating Bells palsy *Ann Otol 78* 1091

Eyzaguirre C 1960 The electrical activity of mammalian intrafusal fibres *J Physiol 150* 169

Fisch U & Esslen E 1970 Zur chirurgische Behandlung der idiopathischen Facialisparese *Med Hyg* (Genève) *28* 1591

Fowler E P Jr 1963 The pathologic findings in a case of facial paralysis *Trans Amer Acad Ophthal Otolaryng 67* 187

Fox J L & Kenmore P I 1967 The effect of ischemia on nerve conduction *Exp Neurol 17* 403

asser H S & Erlanger J 1929 The role of fiber s ze in the establishment of a nerve block by pressure or by cocaine *Amer J Physiol 88* 581

Hilger J A 1949 The nature of Bells palsy *Laryngoscope 59* 228

Jain S N & Sharma A P 1964 Role of compression in the aetiology of Bells palsy An experimental study *J Laryng 78* 266

James J 1961 Ein ge Bemerkungen über die Histolog e des Nervus facialis zur Pathogenese der Bellschen Lahmung *Z Laryng Rhinol Otol 40* 336

Jongkees L B W 1954 On the histology of Bells palsy *Acta Otolaryng* (Stockh) *44* 336

— 1965 Bells palsy A surgical emergency? *Arch Otolaryng* (Chic) *81* 497

— 1968 Surgery of the facial nerve *J Laryng 8?* 575

Kettel K 1947 Bells palsy Pathology and surgery *Arch Otolaryng* (Chic) *46* 427

— 1954 Ischaemic facial palsy Results of decompression of the facial nerve in 108 cases Preliminary report *Acta Otolaryng* (Stockh) Suppl. *116* 155

— 1959 *Peripheral facial palsy* Munksgaard Copenhagen

Kettel K & Jongkees L B W 1963 Sulla technica della operazioni sulla parte endotemporale del nervo facciale *Minerva otorinolaring 13* 97

McGovern G H 1970 Chorda tympani neurectomy for Bells palsy *Arch Otolaryng* (Chic) *92* 189

Miehlke A 1960 *Die Chirurgie des Nervus Facialis* Urban & Schwarzenberg Munchen Berlin

Naumann W H Schliack H & Stuwe J 1968 Indikation zur Facialisdecompression *Deutsch Med Wschr 93* 1308

Romeis B 1968 *Mikroskopische Technik* 16th edition R Oldenburg Verlag Munchen Wien

Sade J 1972 Pathology of Bells palsy *Arch Otolaryng* (Chic) *95* 406

Sade J Levy E & Chaco J 1965 Surgery and pathology of Bells palsy *Arch Otolaryng* (Chic) *82* 594

Sullivan J A & Smith J B 1950 The otological concept of Bells palsy and its treatment *Ann Otol 59* 1148

Sunderland S 1968 *Nerves and nerve injuries* E & S Livingstone Ltd Edinburgh and London

Sunderland S & Cossar D F 1953 The structures of the facial nerve *Anat Rec 116* 147

Taverner D Cohen S B & Hutchinson B C 1971 Comparison of Corticotrophin and Prednisolone in Treatment of Idiopathic Facial Paralysis (Bells Palsy) *Brit Med J 4* 20

Williams H L 1959 Bells palsy *Arch Otolaryng* (Chic) *70* 436

Zulch K J 1970 Idiopathic facial paresis Chapter 17 in *Handbook of Clinical Neurology Diseases of the nerves* Vol 8 Part II (ed P J Vinken & G W Bruyn) North Holland Publishing Company Amsterdam



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# PERIPHERAL FACIAL PALSY IN SUB-ARCTIC NORWAY

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*Abstract* The results of a prospective five-year study of peripheral facial palsy in sub-arctic Norway are reported The idiopathic form accounted for 78 6% of the cases and the incidence for the total population was 18 8/100 000/year This figure is not significantly higher than those reported from several other, more temperate regions The incidence of Bell s palsy was low under 20 years of age, increased markedly in the third decade of life and showed no tendency to falling-off in the elderly No significant seasonal variation was found, nor was there any correlation between age group and season of the year

Idiopathic peripheral facial palsy is a common and distressing clinical problem which has been accorded increased attention in the literature of recent years In spite of intensive clinical and experimental investigation there is still uncertainty as to the incidence, aetiology and preferred mode of treatment Considerable agreement has been reached in the management of certain aetiological groups of peripheral facial palsy, in particular the traumatic, otogenic and herpes zoster paralyses, but the situation with respect to the idiopathic form, or Bell's palsy, which is by far the commonest, is still undecided

It is widely accepted that the idiopathic form of peripheral facial paralysis is caused by an ischaemia of the intra temporal course of the nerve as a result of a raised intra neural pressure secondary to microvascular pathology (Jongkees, 1954, Kettel, 1959), and this theory constitutes the theoretical justification for the operation of decompression Exposure to cold has been frequently cited as a possible cause of reflex ischaemia resulting from dysfunction of the autonomic nervous system (Kettel, 1959, Miehlke, 1960, Zulch, 1970), and this factor has been proposed as a possible explanation for the differing epidemiology of idiopathic facial palsy in different areas of the world (Martin, 1952)

The literature contains comparatively few studies of the incidence of idiopathic facial palsy, and those studies which have been performed present differing results and conclusions The present investigation was therefore instituted to establish the incidence of peripheral facial palsy in a part of the world with a stable population and characterized by rather severe climatic conditions

## MATERIAL AND METHODS

Our hospital serves the northernmost part of Norway, which lies north of the arctic circle and has a population of approximately 177 000 Statistics show that this total is comparatively stable, with an annual population swing of not more than 0 3% in the five-year period 1967–71 In 1966 medical colleagues throughout the region were contacted by letter and requested to refer all patients with peripheral facial palsy for investigation and treatment as soon as the condition was diagnosed Almost all referrals were admitted to the department of otolaryngology

A fixed battery of investigations was per-

Table I *Aetiology of peripheral facial palsy in the five year period 1967 to 1971 (n = 173)*

| | 1967 | 1968 | 1969 | 1970 | 1971 | Total | % |
|---|---|---|---|---|---|---|---|
| Temporal bone fracture | 1 | 4 | 2 | 4 | 3 | 14 | 8 1 |
| Operative trauma | | | 1 | 1 | | 2 | 1 15 |
| Otitis media, acute | | 1 | | | 1 | 2 | 1 15 |
| Herpes zoster cephalicus | 1 | 1 | 1 | | 1 | 4 | 2 3 |
| Parotid tumour | | | | | 1 | 1 | 0 6 |
| Melkersson-Rosenthal syndrome | | 2 | 1 | 2 | 1 | 6 | 3 5 |
| Pregnancy | 1 | | 2[a] | 2 | 3 | 8 | 4 6 |
| Bell s palsy | 12 | 24 | 33 | 39 | 28 | 136 | 78 6 |
| Total | 15 | 32 | 40 | 48 | 38 | 173 | 100 0 |

[a] One patient with polyneuritis gravidarum

formed and a complete neurological examination made The former included a general physical and otolaryngological examination, roentgenological examination of the temporal bones, electrical gustometry and the Schirmer test, audiological and vestibular work ups and fasting blood sugar and herpes zoster antibody estimations Daily percutaneous nerve conduction tests were carried out, and electromyography was performed at regular intervals by the department of neurology

Peripheral facial palsy in pregnancy is not included in the idiopathic material, since this form of facial palsy occurs preferentially at definite stage in pregnancy, may therefore a specific pathogenesis (Mair et al, 5) and also, by definition, occurs only in restricted age group The Melkersson-Rosenthal syndrome, which constitutes a specific clinical complex, is likewise excluded from the Bell s palsy group

All patients diagnosed as having idiopathic peripheral facial palsy, with the exception of a small group (8) of diabetics, received a 10-day course of adrenocorticotrophic hormone

Medical colleagues were contacted at regular intervals throughout the five year period and reminded of our interest in facial palsy

Referral rates in the latter 3 years of the investigation, 1969–1971 were considerably higher than in the first 2, largely on account of an initial reluctance on the part of physicians to send patients the often long distances to our hospital for what is widely considered a "benign" illness The referral rates for the years 1969 through 1971 are therefore considered to give a more accurate index of the incidence of facial palsy in our part of the world

## RESULTS

In the five-year period, 1967–1971, 173 individual peripheral facial palsies were investigated The annual totals for the various aetiological groups are shown in Table I Of the fourteen cases of fracture of the temporal bone, three were of immediate onset and were decompressed, the nerve being found constricted by bone fragments in the pyramidal region The two cases of operative trauma, a revision mastoidectomy and revision parotidectomy, had both received their facial paralysis 10 years earlier at prior surgery, and presented only a temporary deterioration of facial nerve function following reoperation All cases of herpes zoster paralysis were suspected clinically, either on the basis of the dermatological findings, or the presence of auditory and vestibular symptoms, the diagnosis being confirmed by the demonstration of rising antibody titres In no instance was serological evidence of zoster infection found which was not suspected clinically

Of the 134 patients with Bell s palsy, 7 were male and 60 female, 2 females having simultaneous bilateral palsies Fourteen p

Table II *Idiopathic facial palsy, number of cases and incidence rates per 100 000 of the population per year, for both the entire five-year period and for the latter three years of the study, presented as a function of age*

The incidences for the period 1969–71 are presented separately for both sexes in addition to the total incidences

| | 1967–1971 | | 1969–1971 | | | |
|---|---|---|---|---|---|---|
| | | | | Incidence | | |
| Age group | Number | Incidence | Number | Males | Females | Total |
| 0–9 | 7 | 3 9 | 5 | 1 8 | 7 6 | 4 7 |
| 10–19 | 13 | 8 3 | 9 | 6 2 | 13 1 | 9 5 |
| 20–29 | 28 | 19 7 | 22 | 26 2 | 25 4 | 25 8 |
| 30–39 | 22 | 23 0 | 15 | 32 5 | 18 0 | 26 1 |
| 40–49 | 18 | 18 3 | 10 | 19 1 | 14.5 | 17 0 |
| 50–59 | 23 | 24 7 | 18 | 31 3 | 33 2 | 32 2 |
| 60–69 | 12 | 17 8 | 10 | 29 5 | 19 9 | 24 7 |
| 70+ | 13 | 24 8 | 11 | 41 7 | 29 3 | 35 0 |
| 0–70+ | 136 | 15 4 | 100 | 19 3 | 18 4 | 18 8 |

tients (10 5%) had a family history of facial palsy, and 8 (6%), (5 females and 3 males) gave a history of previous episodes either on the same or the contralateral side Thirteen patients (9 7%) had a lingua plicata without otherwise fulfilling the diagnostic criteria for the Melkersson–Rosenthal syndrome, although one of these had previously had a paralysis on the same side

The distribution of the idiopathic facial palsies according to the patients' age is shown in Table II Incidences have been calculated on the basis of population statistics and are expressed as the number of palsies per 100 000 per year These are shown for the entire five year period and separately for the latter 3 years of the study As mentioned previously, the figures for the period 1969–71 are regarded as giving a more accurate estimate of the true incidence of Bell s palsy in this area The total number of palsies and the incidences for the different age groups are presented graphically in Fig 1 for this three year period Incidences are low in the first two decades of life increase markedly after 20 years and show no indication of a falling off in the older age groups In Table II the incidences for Bell s palsy in the period 1969–71 are also shown separately for both sexes There would appear to be a tendency for a higher incidence amongst females in the younger age groups, but the differences are not statistically significant

The seasonal distribution of the 134 Bell's palsies is shown in Table III An ordinary chi square test gives P=0 09, but the Edwards test of cyclic trend demonstrates no correlation between the onset of the palsies

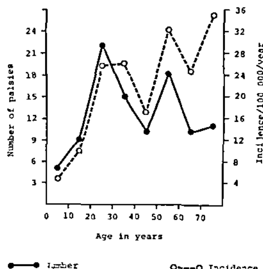



*Fig 1* The number of Bell s palsies and the incidences per 100 000 of the population per year plotted against age groups in decades for the period 1969 to 1971

Table III *Seasonal distribution of Bell s palsy (n = 134, two cases of uncertain onset)*

| | 1967 | 1968 | 1969 | 1970 | 1971 | Total |
|---|---|---|---|---|---|---|
| January | | 1 | 1 | 3 | | 5 |
| February | 1 | 2 | 5 | 8 | 3 | 19 |
| March | | | 3 | 5 | 1 | 9 |
| April | | 2 | 5 | 3 | 1 | 11 |
| May | 1 | 3 | 3 | 5 | 2 | 14 |
| June | | 1 | 2 | 2 | 2 | 7 |
| July | 2 | | 3 | 2 | 3 | 10 |
| August | 1 | 1 | 2 | 2 | 3 | 9 |
| September | 2 | 2 | 3 | 3 | 3 | 13 |
| October | 3 | 4 | 3 | 1 | 3 | 14 |
| November | 2 | 4 | 1 | 3 | 5 | 15 |
| December | | 3 | 2 | 1 | 2 | 8 |
| Total | 12 | 23 | 33 | 38 | 28 | 134 |

and the month of the year On combining the data for age and seasonal distribution (Table IV) no evidence is found that seasonal incidence varies with age group

## DISCUSSION

The term Bell s palsy probably includes several different pathogenetic mechanisms all of which may produce the same clinical picture of a lower motor neurone facial paralysis (Alter 1963 Sade 1972) The facial paralysis of the Melkersson–Rosenthal syndrome pregnancy and herpes zoster infection are neurologically indistinguishable from the group of idiopathic paralyses

In the present material the Melkersson–Rosenthal syndrome accounts for 3 5% of the total number of peripheral facial palsies This figure is surprisingly high when compared with other series in the literature Both in California (Adour & Swanson 1971) and in southern Sweden (Lagerholm & Toremalm 1971) no cases were encountered in comparable series and in Holland only one case was diagnosed in a study of 750 peripheral facial palsies (Devriese & Pelz, 1969) The occurrence of a peripheral facial palsy in a patient with a history of recurrent facial swelling and typical histology is regarded as diagnostic even although lingua plicata is lacking since the latter is found in only approximately one third of cases of this syndrome (Hornstein 1970) It has also been proposed that lingua plicata in the presence of recurrent facial paralysis should be regarded as belonging to the Melkersson–Rosenthal syndrome even though the most important diagnostic feature recurrent facial swelling is absent (Ekbom 1950) These latter criteria were however not applied to the present series

The idiopathic form accounts for between 75% and 85% of all cases of peripheral facial palsy in the literature (Pettersen & Andersen 1967 Lagerholm & Toremalm 1971, Adour & Swanson 1971, Hauser et al, 1971) only Cawthorne (1951) reporting a significantly lower incidence due to the large number of traumatic cases in his series In only one report has Bell s palsy accounted for a significantly higher percentage of cases with 93 6% of patients being classified under this diagnosis (El Ebiary, 1971) but otological examination does not appear to have been performed routinely in this study The present figure of 78 6% is lower than many other reports in the literature but most authors do not differentiate the pregnancy and Melkersson–Rosenthal groups from the general diagnosis of Bell s palsy

The recurrent form is variously reported in from 2 6% (Adour & Swanson 1971) up to 15 2% (Boddie, 1972) of all cases of idiopathic facial palsy The latter author concluded without statistical evidence that the

Table IV *Distribution of Bell s palsy by age group and season of the year*

| | Age group | | | |
|---|---|---|---|---|
| | 0–29 | 30 59 | 60 | Total |
| May–October | 25 | 29 | 13 | 67 |
| November April | 22 | 34 | 11 | 67 |
| Total | 47 | 63 | 24 | 134 |
| June–November | 24 | 31 | 13 | 68 |
| December May | 23 | 32 | 11 | 66 |
| Total | 47 | 63 | 24 | 134 |

Table V *Incidence of recurrent Bell's palsy*

| Author | Recurrent | Total | % Recurrent |
|---|---|---|---|
| Park & Watkins 1949 | 31 | 440 | 7 0 |
| Cawthorne & Haynes, 1956 | 33 | 347 | 9 5 |
| Dalton, 1960 | 5 | 97 | 5 2 |
| Devriese & Pelz, 1969 | 46 | 440 | 10 5 |
| Adour & Swanson 1971 | 8 | 308 | 2 6 |
| Lagerholm & Toremalm, 1971 | 12 | 144 | 8 3 |
| El Ebiary, 1971 | 49 | 528 | 9 0 |
| Boddie, 1972 | 25 | 165 | 15 2 |
| Present series | 8 | 134 | 6 0 |
| Total | 217 | 2 603 | 8 3 |

true incidence of recurrent Bell's palsy was probably less than 5% A review of several series in the literature (Table V) gives a mean incidence of 8 3% for recurrent idiopathic facial palsy Devriese & Pelz (1969) reported a *statistically significant greater recurrence* rate in males, whereas El Ebiary (1971) came to the diametrically opposite conclusion Unfortunately, the sex distribution of recurrent Bell's palsy is not mentioned in the majority of reports in the literature

Idiopathic facial palsy is generally considered a disease of the younger, middle age group, the decades from 20 to 50 showing the highest incidence (Park & Watkins, 1949, Cawthorne & Haynes, 1956, Taverner, 1959, Gregg 1961, Lagerholm & Toremalm, 1971) A strikingly different distribution has been reported by El Ebiary (1971) from Alexandria, where 44 8% of cases occurred in the age *group younger than 20 years* However, in none of these studies was the incidence correlated with the age distribution of the population under consideration The present material demonstrates that the incidence in northern Norway is lowest in the first two decades, increases markedly after 20 years and remains at a high level thereafter Only Hauser et al (1971) have treated their data in a similar manner and found a strikingly similar age distribution In agreement with the report of Alter (1963) the present study shows a slightly greater incidence amongst females in the younger age groups, but this difference is not statistically significant

Epidemiological studies which are based on the referral of cases to a central, specialty department are notoriously unreliable, as evi*denced by the differences between our three-* and five year statistics The incidence of 18 8/100 000/year for the three-year period 1969–1971 in northern Norway is of a similar order of magnitude as several other reports in the literature (Table VI) A common rate of 15/100 000/year is obtained when the data for the six regions included in Table VI are combined The report from Sweden (Lagerholm & Toremalm, 1971) gives a significantly lower incidence ($X^2$ 10 09), whilst the series from Minnesota (Hauser et al, 1971) is significantly higher ($X^2$ 26 49) than the calcu-

Table VI *Incidence of Bell's palsy in various regions*

| Region | Author | Incidence | Study | Observed | Expected | $\chi^2$ |
|---|---|---|---|---|---|---|
| Belfast | Gregg | 16 0 | 4 400 | 661 | 660 0 | 0 00 |
| England | Melotte | 16 6 | 165 | 29 | 24 8 | 0 71 |
| California | Adour & Swanson | 13 5 | 1 800 | 243 | 270 0 | 2 70 |
| Sweden | Lagerholm & Toremalm | 11 5 | 1 250 | 144 | 187 5 | 10 09 |
| Minnesota | Hauser et al | 22 8 | 507 | 121 | 76 1 | 26 49 |
| North Norway | Mair & de Graaf | 18 8 | 531 | 100 | 79 7 | 5 17 |
| Total | | | 8 653 | 1 298 | 1 298 1 | 45 16 |

Common rate 1 298/8 653 × 100 = 15 0 100 000 year

Study is the product of the number of years and the population in thousands in each series Observed and Expected refer to the number of palsies in each study

Table III *Seasonal distribution of Bell's palsy (n = 134, two cases of uncertain onset)*

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Leibowitz, U 1966 Bell s palsy—two disease entities? *Neurology* (Minneap) *16* 1105

Mair, I W S, Elverland, H H & Johannessen, T A 1973 Idiopathic facial palsy and pregnancy *Ann Otol 82*, 235

Martin R C. 1952 Bell s palsy *Arch Otolaryng* (Chic ) *55*, 405

Melotte, G 1961 Idiopathic paralysis of the facial nerve *Practitioner 187* 349

Miehlke, A 1960 *Die Chirurgie des Nervus facialis* Urban & Schwarzenberg, Munich-Berlin

Park, H W. & Watkins, A L 1949 Facial paralysis *Arch Phys Med 30*, 749

Petersen, E & Andersen, P 1967 Spontaneous course of 220 peripheral, non traumatic facial palsies. *Acta Otolaryng* (Stockh ), Suppl *224* 296

Sadé, J 1972 Pathology of Bell s palsy *Arch Otolaryng* (Chic ) *95* 406

Taverner, D 1959 The prognosis and treatment of spontaneous facial palsy *Proc Roy Soc Med 52*, 1077

Zulch, K J 1970 Idiopathic" facial paresis *Handbook of clinical neurology* (ed P J Vinken & G W Bruyn), vol 8, Part 11 North Holland Publishing Company, Amsterdam

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# MALIGNANCY OF ACINIC CELL TUMOURS ELUCIDATED BY MICROSPECTROPHOTOMETRIC DNA ANALYSIS

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*Abstract* "Acinic cell tumour" is nowadays generally considered as a biological malignant tumour type but the often existing pronounced benign histological features have raised the question unless beside the malignant tumours of this type there also exists a benign variety In order to study this problem microspectrophotometric determinations of the nuclear DNA content of Feulgen stained cells of an acinic cell tumour without malignant histological features have been performed and compared with control cells and tumour cells from a biological benign and a biological malignant salivary gland tumour The microspectrophotometric DNA analysis performed in the present study showed that the nuclei of an acinic cell tumour with histologically benign features exhibited signifi[illegible]y higher DNA values than normal control cells [illegible] the cells from the benign tumour (a monomorphic adenoma) On the other hand the microspectrophotometric analysis showed that the nuclear DNA content of the "acinic cell tumour" cells was increased to the same level as in the cells of a poorly differentiated adenocarcinoma Thus the results of the DNA analysis argue against the existence of a benign variety of the acinic cell tumour which therefore irrespective of the histological features should be considered as a malignant type of tumour

Until 1953 when Buxton et al showed that "acinic cell tumour" could have a clinically malignant course its histologically appearance led to its being denoted as a benign type This is evident from the various synonyms in the literature used for it such as "epithelioma glandulaire" (Masson 1924) "acinare Adenome" (Lang, 1929) "parathyreoidea ähnliche Geschwulst" (Franssen 1932 33 Huckel 1930) "serous acinar adenoma" (Skorpil 1940) "glycogen rich clear-cell adenoma (Corridan 1956)

The cytological picture is often dominated by cells denoted as acinic cells because of their great resemblance to normal acinar cells In view of the presumed benign course and the dominant type of cell the name "acinic cell adenoma" was introduced (Godwin & Colvin 1948) Studies during the past 15 years however, have disclosed that the "acinic cell tumour" can metastasize and cause death in the tumour disease The terms "acinic cell adenocarcinoma" (Godwin et al, 1954) and "acinic cell carcinoma" (Beahrs et al, 1960 Grage et al, 1961) therefore were proposed.

In "acinic cell tumours' the often pronounced benign morphological features have raised the question, whether there is a benign variety of this tumour Thus, Kleinsasser et al (1967) have stated that there exist undoubtedly benign and malignant forms of acinic cell tumour, but that it is not possible on the basis of histological features to separate the benign and malignant types

Seifert (1966) combined "acinic cell carcinoma" with "acinar adenoma' and "alveolar adenoma" in a group which he called "Acinuszelltumoren' and denoted the group as "fakultativ maligne" Kleinsasser et al and other authors who believe in a benign and a malignant form of this tumour type use the designation

"acinic cell tumour" whereas authors who believe that there is no definitely benign group of this tumour denote it as "acinic cell carcinoma"

Increased nuclear DNA content has been shown to be a characteristic feature of malignant salivary gland tumour cells (Eneroth & Zetterberg, in press) In order to add information to the question of malignancy in acinic cell tumours with histologically benign structures an analysis of the nuclear DNA content in the individual cell nuclei of an acinic cell tumour without malignant histological features has been performed

## METHODS

A parotid tumour with histological structures typical for an acinic cell tumour without malignant histological structures was selected for the analysis For the microscopic examination of the tumour routine histological techniques were employed

For the microspectrophotometric analysis of the nuclear DNA content imprint preparations were made from the cut fresh surface through the tumour tissue immediately after the removal of the tumour Haemacytometer glass slides were used The imprint preparations were immediately fixed in a freshly prepared mixture of ethanol and aceton (1 1) for 30 minutes and thereafter stored in the refrigerator (+4°C) until the staining was performed according to the Feulgen procedure (Eneroth & Zetterberg, in press) Human lymphocytes from peripheral blood and cells from normal parotid gland were used as control cells Furthermore cell populations from a biological benign salivary gland tumour (a cell rich monomorphic adenoma) and from a clearly malignant salivary gland tumour (a poorly differentiated adenocarcinoma) were microspectrophotometrically analysed for comparison The DNA content of the Feulgen-stained individual cell nuclei was determined by absorption measurements in a rapid scanning microspectrophotometer (Lomakka, 1965) at the wavelength of 546 nm

## RESULTS

The histological slides were studied by the following methods of staining hematoxylin eosin, van Gieson and PAS

The tumour, that was macroscopically circumscribed and measured 2 cm in diameter, was built up by round and polyhedral cells arranged in equally sized lobules separated by thin septa of connective tissue (Fig 1) No evident glandular lumina were observed

The histological pattern of the tumour was consistent with an acinic cell tumour The only differential diagnosis considered was that of an oncocytoma The cytoplasms of the tumour cells were, however, mostly granular and faintly basophilic, and in small areas there also existed clear cells PAS-positive material was found in many cells, strongly indicating the "acinic cell tumour" diagnosis

The tumour was mostly monomorphous without cellular atypia but in some parts one could find a slight degree of nuclear abnormalities in the form of single, somewhat enlarged hyperchromatic nuclei Histomorphological criterias of malignancy were, however, lacking No obvious invasion could be demonstrated

Microspectrophotometric measurements of randomly selected Feulgen stained nuclei of the imprint preparations are illustrated in Fig 2 As control cells for the Feulgen staining procedure human lymphocytes from peripheral blood were used The mean Feulgen value of the human lymphocytes was given the value 2c, which denotes the normal diploid DNA content Each measured value was related to its corresponding staining control and expressed in the 2c units It is clear from the figure that the nuclei from the "acinic cell tumour" contain more DNA than the lymphocytes, the normal parotid gland cells and the benign monomorphic adenoma cells, which all



*Fig. 1.* Acinic cell carcinoma of the parotid gland. Photomicrograph × 400.

exhibit the normal diploid DNA content, 2c. On the other hand the similarity between the "acinic cell tumour" and the clearly malignant poorly differentiated adenocarcinoma from the parotid gland is evident, the majority of the nuclei from these two tumour types contain between 2.2c and 2.5c units of DNA, i.e. a clearly hyperdiploid value.

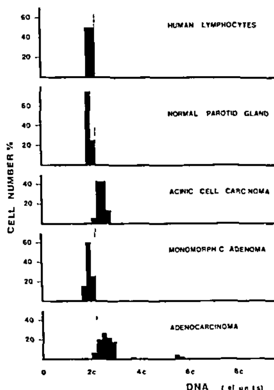



*Fig* 2 Frequency histograms of nuclear DNA quantity (Feulgen positive material) Five different cell types were analysed human lymphocytes from peripheral blood (staining controls) cells from a normal parotid gland from an acinic cell tumour from a monomorphic cell rich adenoma of the palate and from a poorly differentiated adenocarcinoma of the parotid gland Each histogram is based on the microspectrophotometric analysis of between 50 and 75 randomly selected cells The DNA values are expressed in relation to the staining control (human lymphocytes) which was given the value of 2c

## DISCUSSION

The microspectrophotometric DNA analysis performed in the present study showed that the cell population of an acinic cell tumour with histologically benign features exhibited significantly higher DNA values than normal control cells and the cells from a benign tumour (a monomorphic adenoma) Despite the histologically benign features of the acinic cell tumour in the present study, the microspectrophotometric analysis showed that the nuclear DNA content of the tumour cells was increased to the same level as in the cells of a clearly malignant salivary gland tumour (a poorly differentiated adenocarcinoma) These two tumours both had a modal DNA value between 2 2c and 2 5c, suggesting the existence of an aneuploid stemline in the hyperdiploid region

From a clinical follow up study of a large tumour material for a long period (Blanck et al, 1971) it is obvious that the highly differentiated mucoepidermoid tumour and the acinic cell tumour are more low-grade malignant than the other malignant salivary gland tumours The existence of a benign tumour variety of these two tumour types has also been questioned Concerning mucoepidermoid tumours with highly differentiated structures Eneroth & Zetterberg (1973) have shown that this tumour type exhibits higher DNA values than normal control cells and should despite the benign histological features be considered as malignant and therefore be denoted as a carcinoma Concerning the acinic cell tumour without histologically malignant features the present study indicates that also acinic cell tumour despite the benign histological features exhibits DNA values characteristic of cells of a clearly malignant salivary gland tumour These facts argue against the existence of a benign variety of the acinic cell tumour Thus, the acinic cell tumour irrespective of the histological features, should be considered as malignant and therefore be denoted as *'acinic cell carcinoma'*

## ZUSAMMENFASSUNG

Ein ‚Acinuszelltumor' wird nunmehr allgemein als ein biologisch maligner Tumortyp angesehen aber die oft vorliegenden ausgesprochen benignen histologischen Strukturen führen zu der Frage ob neben den malignen Tumoren dieses Typs nicht auch eine benigne Variante besteht Zum näheren Studium dieses Problems wurden mikrospektrophotometrische Bestimmungen des nuklearen Gehalts an DNS bei Feulgenscher Nuklealfärbung eines Acinuszelltumors ohne maligne histologische Strukturen durchgeführt und mit Kontrollzellen und Tumorzellen eines biologisch benignen und eines biologisch malignen Speicheldrüsentumors verglichen Die mikrospektrophoto-

metrische DNS-Analyse zeigte dass die Nuklei eines Acinuszelltumors mit histologisch benignen Strukturen signifikant hohere DNS-Werte aufwiesen als normale Kontrollzellen und die Zellen des benignen Tumors (eines monomorphen Adenoms) Andererseits zeigte die mikrospektrophotometrische Analyse dass der nukleare DNS Gehalt der Zellen des Acinuszell tumors im gleichen Ausmass erhoht war wie in den Zellen eines gering differenzierten Adenokarzinoms Die Resultate der DNS-Analyse widersprechen somit der Existenz einer benignen Variante des Acinuszell tumors Ein solcher Tumor muss daher ohne Ruck sicht auf die histologischen Strukturen als ein ma ligner Tumortyp angesehen werden

## REFERENCES


Beahrs O H Woolner L B Carveth S W & Devine K D 1960 Surgical management of parotid lesions Review of 760 cases *Arch Surg* (Chic) *80* 890

Blanck C Eneroth C M & Jakobsson P A 1971 Mucus producing adenopapillary (non epidermoid) carcinoma of the parotid gland *Cancer 28* 676

Buxton R W Maxwell J H & French A J 1953 Surgical treatment of epithelial tumours of the parotid gland *Surg Gynec Obstet 97* 401

Corridan M 1956 Glycogen rich clear cell adenoma of the parotid gland *J Path Bact 72* 623

Eneroth C M & Zetterberg A 1973 Nuclear DNA content as a criterion of malignancy in salivary gland tumours of the oral cavity *Acta Otolaryng* (Stockh) *75* 296

— Microspectrophotometric DNA analysis of malig nant salivary gland tumours *Acta Otolaryng* (Stockh) In press

anssen R 1932 33 Eine Parothyreoidea ahnliche Geschwulst der Ohrspeicheldruse *Zbl Allg Path 56* 113

Godwin J T & Colvin S H 1948 Adenoma of the parotid gland *Arch Path* (Chic) *46* 187

Godwin J T, Foote F W & Frazell F L 1954 Acinic cell adenocarcinoma of parotid gland Re port of 27 cases. *Amer J Path 30* 465

Grage T B, Lober P H & Arhelger S W 1961 Acinic cell carcinoma of the parotid gland A clinicopathological review of eleven cases *Amer J Surg 102* 765

Huckel R 1930 Eine ungewohnliche Adenomform der Parotis *Verh Deutsch Ges Path 25* 342

Kleinsasser O Hubner G & Klein H J 1967 Acinuszelltumoren der Glandula parotis *Arch Ohr Nas Kehlkopfheilk 189* 33

Lang F J 1929 Epitheliale Geschwülste der Speichel drusen I Adenom und Zystadenom In *Handbuch der speziellen pathologischen Anatomie* (ed F Henke & O Lubarsch) Bd 5 Springer Berlin

Lomakka G 1965 A rapid scanning and integrating cystophotometer *Acta Histochem* (Jena) Suppl 6 47

Masson P 1924 Tumeurs des glandes annexes des muqueuses de la face et du cou *Atlas du Cancer* III et IV Ed de L Ass Fr p l Et d Cancer

Seifert G 1966 Mundhohle Mundspeicheldrusen Tonsillen und Rachen In *Spezielle pathologische Anatomie* (ed W Doerr & E Uehlinger) Bd 1 Springer Berlin Heidelberg and New York

Skorpil F 1940 Über das Speicheldrusenadenom *Virchows Arch Path Anat 306* 714



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# THE EFFECTS OF SYMPATHETIC NERVE STIMULATION ON THE TRACER DISAPPEARANCE RATE AND LOCAL BLOOD CONTENT IN THE NASAL MUCOSA OF THE CAT

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*Abstract* The effects of sympathetic nerve activation on tracer disappearance rate and local blood content in the cat nasal mucosa were studied by measuring local disappearance of $^{125}I^-$ and changes in gross pulse rate from $^{131}I$ labelled serum albumin monitored over the nose Major changes in the tracer disappearance rate were seen in the frequency range of 0 5–6 0 imp/sec while similar changes in the local blood content occurred at 0 1–0 5 imp/sec indicating a functional differentiation in the vasoconstrictor control of the exchange and capacitance vessels during low sympathetic tone The observed responses were shown to be due to activation of sympathetic adrenergic nerves and receptors of the α type No evidence of vasodilator influence due to stimulation of β receptors or sympathetic cholinergic vasodilator fibers was found

The effects of sympathetic nerve activation on the local blood content of the nasal vascular bed has previously been studied with rhinomanometric techniques, where changes in the lumen of the nasal passages yielded indirect measurements of changes in the blood content (capacitance function) of the mucosa (Tschalussow, 1913, Malcomson, 1959, Franke, 1966, Hall & Jackson 1968, Richerson & Seebohm, 1968, Rooker & Jackson, 1969) These experiments suggest a strong sympathetic influence on the capacitance vessels although a direct method for the study of the capacitance function has so far not been demonstrated

Little is known about the type of sympathetic receptors in the nasal mucosa and no information is available on the sympathetic influence on the exchange vessels in the nasal vascular bed Therefore, knowledge of the interaction between events in the exchange and capacitance vessels is still not at hand The present investigation was undertaken to study these events as reflected in the effects of graded sympathetic nerve activation on tracer disappearance rate and local blood content in the nasal mucosa We also wanted to classify the receptors mediating the vascular responses to sympathetic nerve activation It might be observed that in procedures providing quantitative information about changes in the vascular bed, the various vascular sections should preferably be studied simultaneously (cf Mellander & Johansson, 1968)

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### *Surgical Procedures*

The experiments were performed on 49 cats (2 4–4 3 kg) anesthetized with chloralose urethane (50 mg/kg + 100 mg/kg i v ) or, in three cases where denervation effects were studied, chloralose (60 mg/kg i v ) after induction with ether The trachea was cannulated The pressure in the femoral artery was measured by a Statham pressure transducer (P 23A) and recorded on a Rikadenki multichannel recorder Rectal temperature was measured and was kept constant at 38°C by heating lamps The cervical sympathetic trunk was dissected free

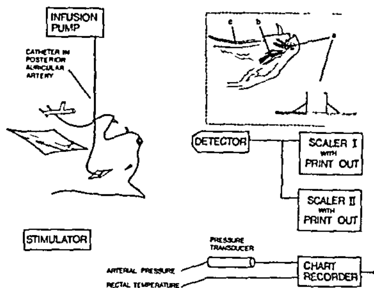



*Fig 1* Drawing showing experimental procedure The lead shields masking the nasal cavities are not shown Inset represents sagittal section of the head of the cat and the application technique with the polyethylene tube fixed over the de epithelized area on the inferior concha (*a*) middle concha (*b*) naso pharynx (*c*)

from the vagal nerve and transected Sympathetic stimulation was performed in the cranial direction using a bipolar silver electrode The stimuli were monophasic square wave pulses delivered by a Grass model S4 stimulator Stimulation frequencies were varied between 0 1–12 imp/sec in random order In the experiments where α-adrenergic blocking agents, isoproterenol and papaverine were used, the posterior auricular artery was cannulated for close intra-arterial infusion into the external carotid artery The head of the cat was immobilized by means of a steel rod inserted between the jaws and secured in place by dental acrylic

### *Disappearance Measurements*

The tracer disappearance measurements were performed with an easily diffusible water-soluble radioactive tracer, $^{125}$I, as iodide (Kety, 1949) The tracer was obtained as an isotonic, carrier free solution in concentrations of 300 μCi μl (AB Atomenergi Nykoping, Sweden) In preliminary experiments volumes of 0 5–1 5 μl *of the isotope were injected subepithelially* in the mucosa of the anterior two-thirds of the inferior concha on one side The mucosa was visualized by widening of the vestibulum of the nose with a speculum and inspection through a Leitz operation microscope A glass capillary with a tip diameter of 50–100 μm was used as an injection cannula The capillary was attached with a polyethylene tube (PE 20) to a Hamilton 10 μl syringe, fixed to a stand The injection time was 3–5 min In 2 cats the radioactive tracer was injected together with an intravital dye (5% Fluorescein sodium, or 6% Procoin navy blue) and the injection area was examined histologically to evaluate the spread of the tracer In a second series of experiments using 15 cats the injection technique was changed to an application technique The epithelium at the application site was removed and a small polyethylene tube (PE 90) applied and fixed over the exposed area with an adhesive (Nobecutan, Bofors), thus creating an open well with the subepithelial tissues in the bottom (Fig 1) About 1 hour later 0 5–1 μl of the tracer was placed in the cavity The disappearance of the applied depot was monitored by an external scintillation detector secured in a fixed relation to the nose The distance between the depot and the detector was 5–15 cm Apart from the nose, the animal *was shielded by 3 mm lead plates The detector* output was fed into two recording channels each containing a single channel pulse height analyzer and two scalers with digital print out A schematic drawing of the setup is shown in Fig 1 For further technical details concerning

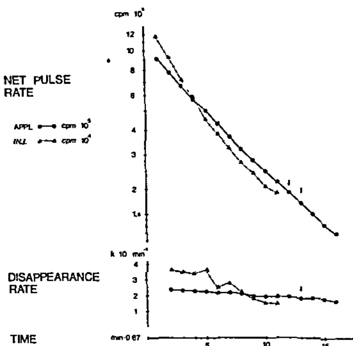



*Fig 2* Disappearance rates of a ¹²⁵I-depot injected subepithelially and a locally applied depot in the same area Resting conditions The disappearance rate is illustrated in two ways, both as a semilogarithmic plot of the net pulse rate of the depot against time and as *k* values taken from the slopes between two consecutive readings The *k* value at the arrow was derived from the slope between the two points in the net pulse rate curve indicated with arrows

the apparatus, see Bolme & Edwall (1970) Radioactivity was counted for periods of 40–60 sec, and measurements started immediately after the application of the tracer After each run the total final background was determined (cf Odeblad et al, 1959) The net pulse rate (gross pulse rate minus background) was plotted semilogarithmically against time The disappearance rate (*k*-value) which represents the fractional elimination of the depot per minute was calculated as follows (Kety, 1949)

$$k \quad (\log C_1 - \log C\ )/0\ 4343\ (t_2 - t_1)$$

where $C_1$ and $C_2$ are the recorded net pulse rates per minute of the depot at the time $t_1$ and $t_2$ The calculations were made on a computer for each time interval For further details concerning the calculations see Edwall (1971)

Reduction in tracer disappearance rate ($k$ %) was calculated from the expression $k\% = 100\ (k_{contr}\quad k_{stim})/k_{contr}$ where $k_{contr}$ and $k_{stim}$ are the means of four *k*-values during the preceding control period and the stimulation period, respectively

### *Measurements of Local Blood Content*

In 7 cats, changes in total blood volume of the nose were studied separately or simultaneously with the disappearance measurements A lead shield of 5 cm thickness fitting tightly around the head and the nose of the cat had a narrow slit over the right side of the nose and shielded the rest of the cat from the detector ¹³¹I-albumin (1–2 5 mCi in 5 ml) was injected, intravenously Gross pulse rate was measured as described above When double tracers were used, the pulse rates in both recording channels were determined separately Appropriate corrections were made for the overhearing of ¹³¹I in the ¹²⁵I channel Reductions in gross pulse rate during stimulation were expressed as a percentage of the maximal reduction obtained at 6 imp/sec

## RESULTS

### *Tracer disappearance measurements during resting conditions*

Following injection of the tracer solution into the mucosa the disappearance rate (*k* value) varied considerably in different injections

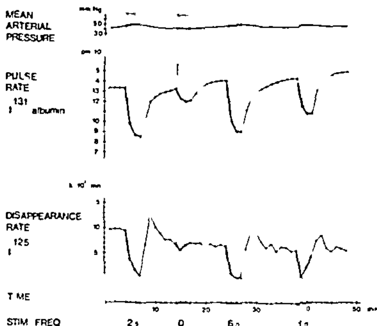



*Fig 3* Influence of sympathetic nerve stimulation on the disappearance rate from a locally applied depot of $^{125}$I and the gross pulse rate of $^{131}$I albumin measured over the right nasal cavity

The deep injections or injections where a slight bleeding occurred regularly resulted in a polyexponential disappearance curve with an initially high disappearance rate during the first 5 10 min which thereafter decreased almost monoexponentially down to a final background Results from these curves could only be evaluated qualitatively as changes occurred in the disappearance rate even under resting conditions

Subepithelial injections resulted in an almost monoexponential disappearance curve with a high disappearance rate for about 10 min down to the background (Fig 2) This curve was most often found after the first injection or when the thin epithelial layer was seen to bulge slightly during the injection Histological examination of the mucosa after a corresponding injection of an intravital dye revealed a subepithelial spread of the depot and penetration down into the submucosa No penetration through the epithelial layer could be seen

A comparison between a subepithelially injected depot and a depot applied immediately afterwards at the same site is shown in Fig 2 The $\lambda$ value was initially higher in the injected depot than that from the local application but gradually decreased

In the experiments where the application technique was used (15 cats 72 applications) the disappearance curves showed a rapid mono exponential disappearance rate for 15 30 min and came down to a low final background recording which was not changed noticeably when the cavity was rinsed with saline When the application was made immediately after the preparation a slight decrease in the $\lambda$ values was noted as seen in Fig 2 where the $\lambda$ value decreased from 0 23 to 0 17 in 11 min

*Local blood content during resting conditions*

Provided that no bleeding occurred within the area seen by the scintillation detector no noticeable change occurred in the gross pulse rate during a 30 50 min period

*Effect of sympathetic nerve stimulation*

In all experiments sympathetic nerve stimulation in the frequency range of 0 1–6 imp sec induced frequency dependent reductions in the $\lambda$ value and local blood content

Fig 3 shows an experiment where both tracers were studied simultaneously Stimulation at 2 5 imp/sec reduced the disappearance rate by 83% while the subsequent low fre

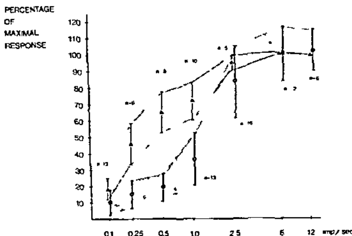



*Fig 4* Influence of sympathetic nerve stimulation on tracer disappearance rate (shaded curve) and the local blood content (unshaded curve) in the nasal mucose Changes expressed as percent of the reduction at 6 imp sec Mean standard deviation and number of observations are shown

quency stimulation at 0 1 imp/sec induced a reduction of 14% Stimulation at 6 imp/sec reduced the disappearance rate by 90% During the subsequent recovery after stimulation frequencies above 1 imp/sec there was a transient overshoot followed by return to control values At frequencies of 1 imp/sec or below an escape was seen

The gross pulse rate of $^{131}$I albumin was simultaneously reduced and a maximal reduction was seen at 6 imp/sec and a clearcut reduction at 0 1 imp/sec which was 25% of the maximal reduction No escape was found in any of the experiments When oximetazoline (Nezeril, Draco) was applied to the mucosa and compared with sympathetic stimulation it was found that the reduction in pulse rate was 80–90% of that following supramaximal stimulation

The relationship between stimulation frequency and the percentage change in *k*-values from disappearance measurements with the application technique is demonstrated in Fig 4 (shaded curve) It is seen that a frequency-dependent response was observed between 0 5 and 6 imp/sec

The responses to repeated stimulation with the same frequency varied less within the same animal than between animals The same qualitative results were also obtained in disappearance measurements from the locally injected depots

The effect of sympathetic nerve stimulation on the local blood content is also illustrated in Fig 4 A pronounced increase in the magnitude of the responses was observed between 0 1 and 0 5 imp/sec The decrease in the pulse rate was 22, 55 and 75% at 0 1, 0 25 and 0 5 imp/sec respectively An almost maximal response was obtained at 2 5 imp/sec Comparing the frequency response curves for tracer disappearance rate and local blood content (Fig 4), it is seen that the frequency response curve for the local blood content is shifted to the left in relation to the corresponding curve for the disappearance rate

### *Sympathetic stimulation after an α-adrenergic blocking agent*

After infusion of dihydroergotamin (Orstanorm, Sandoz, 0 2 mg kg) in 2 cats the effects of stimulation at frequencies below 1 imp/sec were almost completely abolished At higher stimulations a partial block was found Phentolamine 5 mg/kg (Regitine, Ciba) was infused in four experiments and resulted in a complete block at all stimulation frequencies

In order to investigate the presence of β receptors the α receptors were blocked Stimulation after a complete block did not induce any significant increase in the disappearance rate (Fig 5 3) Infusion of a β adrenergic agent, isoproterenol, in doses of 0 01 μg–0 30 μg/min also failed to influence the disappear-

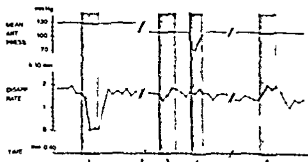



*Fig 5* Influence of an α adrenergic blocking agent on disappearance rate of ¹²⁵I during sympathetic stimulation and the effects of isoproterenol and papaverine Cat 3 2 kg

*1* Stimulation 6 V 1 msec 12 imp/sec
*2* Phentolamine i a infusion 5 mg/kg
*3* Stimulation 6 V, 1 msec 12 imp/sec
*4* Isoproterenol i a infusion 0 30 $\mu$g/min
*5* Papaverine i a infusion 0 30 mg/min

ance rate The latter dose caused a fall in the systemic blood pressure which can be seen in Fig 5 4

The response in the local blood content upon sympathetic stimulation could also be blocked by phentolamine as seen in Fig 6 A partial blockade was seen after infusion of 2 5 mg phentolamine while a complete block was obtained even at high frequencies after 5 mg/kg No increase in the local blood content was then noted upon repeated stimulation

*Acute effects of sympathetic denervation*

To obtain information about the influence of sympathetic nervous tone during resting conditions the sympathetic nerve was transected during disappearance measurements on 4 cats and during measurements of local blood content in 1 cat Such denervation consistently resulted in a slight increase (Maximally 16%) in the rate of tracer disappearance ($\lambda$-value) and an increase in the local blood content

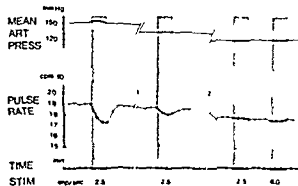



*Fig 6* Influence of an α adrenergic blocking agent on the gross pulse rate of ¹²⁵I albumin during sympathetic stimulation Cat 2 5 kg *1* Phentolamine 2 5 mg i a infusion *2* Phentolamine 10 mg i a infusion

## DISCUSSION

No attempt has been made to transform the disappearance rate ($\lambda$-value) of iodide into values of total blood flow since other factors, such as changes in capillary exchange surface area and capillary flow distribution and velocity, are of quantitative importance (cf Mellander & Johansson, 1968) Furthermore, the abundant arterio-venous anastomoses within the nasal vascular bed as described by Dawes & Prichard (1953) would invalidate calculations of total blood flow based on $\lambda$-values since these are dependent on blood flow through the exchange vessels (Kety, 1960) For example, a redistribution of blood flow from shunt vessels to exchange vessels without changing total blood flow would increase the $\lambda$ values

Among other factors that may influence the disappearance rate is the trauma induced by the injection needle, and the injected volume (Sejrsen, 1971) Though the present technique was aimed at reducing these factors, the rapid initial disappearance rate seen after the deep injections is probably a result of the injection trauma and possibly also a consequence of heterogenous tissue perfusion within the depot (cf Edwall & Kindlova, 1971) The slow disappearance rate seen during the monoexponential late part of these curves might be due to diffusion barriers within the depot The application technique was developed to overcome the difficulties with the injection technique The slight decrease in the $\lambda$ values seen at local application immediately after the preparation is probably also the effect of trauma since this effect was not noticed when the application was made 1 hour later A possible

reason for the monoexponential disappearance curve regularly found with the application technique could be the existence of rate limiting barriers for diffusion between the depot and the exchange vessels However, as increases in disappearance rate were found when the blood vessels were dilated with papaverine, this limitation would then only exist at higher flow rates

Using $^{131}$I labelled albumin we also obtained data on the local blood content of the nasal mucosa Rhinomanometry has previously been used to record changes in the lumen of the nasal passages and thus indirectly measure changes in the blood content of the mucosa An assumption important for this technique is that the quantity of interstitial or intracellular fluid is not altered at the same time, or measurements are not disturbed by excessive nasal secretion (cf Drettner, 1963) With the method described here these difficulties are avoided and the measured radioactivity expresses relative changes in plasma volume in the tissues seen by the detector If it is assumed that the hematocrit is not changed during the experimental procedures, then the measured changes represent changes in the regional blood volume Jodal & Lundgren (1970) studying intestinal blood volume distribution, did not find any significant change in the relative distribution of red cells and plasma volumes during nervous vasoconstriction and it seems reasonable to assume a similar relationship in the nasal vascular bed

To what extent measured changes represent changes solely in the nasal vascular bed is difficult to state with certainty and depends on whether the detector monitors events from surrounding tissues However, since the local application of a vasoconstrictor drug on the nasal mucosa induced a reduction which was 80–90% of that obtained following maximal sympathetic nerve stimulation, it appears likely that most of the changes would reflect alterations in the blood content (capacitance function) of the nasal mucosa

The maximal effects of sympathetic nerve stimulation on tracer disappearance rate, corresponding to around a 70% reduction of the $k$-values, were obtained with frequencies of about 6 imp/sec This magnitude of change is similar to that seen in oral tissues and skeletal muscle as reported by Edwall & Kindlova (1971), who used similar tracer disappearance techniques

The major changes in the exchange function were seen in the frequency range of 0 5–6 0 imp/sec while similar changes in the capacitance function occurred already at 0 1–0 5 imp/sec This effectiveness of low frequency stimulation on the capacitance function is in agreement with results obtained with a rhinomanometric technique in the dog (Franke, 1966)

Our results showing a dissociation in sympathetic response between the exchange and capacitance function might be explained by the very rich adrenergic innervation around the venous sinusoids and the less pronounced innervation within the subepithelial and periglandular vascular plexus as found in histochemical studies of the adrenergic innervation of the nasal mucosa (Dahlstrom & Fuxe, 1967, Änggard & Densert, to be published)

A relative difference in vasoconstrictor response between the resistance and capacitance vessels of the cats leg has further been described by Mellander (1960) and was partly explained by the difference in the wall/lumen ratio of the resistance (mainly thick-walled arterial vessels) and the capacitance vessels (mainly thin walled veins) This explanation might also apply to the nasal mucosa where wide thin walled veins occur abundantly

Furthermore, the shift to the right of the frequency–response curve for the tracer disappearance rate could also be explained by a redistribution of blood flow from shunts to exchange vessels, since shunts in skin have been shown to be highly sensitive to low sympathetic vasoconstrictor activity (cf Mellander & Johansson 1968)

Hall & Jackson (1968), in a rhinomanometric study of the effects of α- and β adrenergic

agonists, demonstrated the presence of α receptors in the nasal mucosa while the existence of β receptors was considered to be doubtful. In the present study the effects of α-receptor blocking agents clearly demonstrate that the observed responses in the exchange and capacitance vessels are due to activation of sympathetic adrenergic nerves and receptors of the α type. When a complete blockade of the α-receptors was obtained, no increase in the exchange or capacitance function was observed during sympathetic nerve stimulation. Hence, there is no evidence for a vasodilatator influence due to stimulation of β receptors or sympathetic cholinergic vasodilator fibers in the nasal vascular bed. Ishii & Toriyama (1972) observed in a histological study of the human nasal mucosa a cholinergic innervation independent of the sphenopalatine ganglion. This observation, if applicable to the cat nasal mucosa, would then not represent sympathetic cholinergic vasodilator fibres affecting the exchange- or capacitance vessels. Cutting the cervical sympathetic nerve did not increase the disappearance rate more than 16% indicating a resting impulse frequency of less than 0.5 imp/sec during the present conditions. This low sympathetic tone might be attributed to the narcosis as suggested by Rooker & Jackson (1969) because in the present study, a hyperaemia of the external nose was noted during the induction of the narcosis.

Experiments on animals and humans have shown that the tonic discharge rate of autonomic sympathetic fibers under resting conditions is about 1–3 imp/sec and rarely exceeds 10 imp/sec (Folkow 1952, Folkow & Hamberger, 1956). Our results indicate that even small variations within this frequency range would elicit marked changes in the exchange function and cause an almost maximal constriction of the capacitance section. At discharge rates below 0.5 imp/sec major changes would occur only in the capacitance section. The marked effectiveness of low frequency stimulation on the capacitance function in the nasal mucosa as found in the present investigation, might indicate that nasal airway patency mainly is influenced by changes in sympathetic tone as previously suggested by Richerson & Seebohm (1968). The exchange function at those low frequencies, however, seems only moderately affected. To what extent this function is affected by changes in parasympathetic tone is however open to question and will be the subject of further studies.

## ZUSAMMENFASSUNG

Der Effekt der sympathischen Nervaktivierung auf die Abklingquote von radioaktivem Material und das lokale Blutvolumen wurde an der Nasenschleimhaut der Katze untersucht. Es wurden die lokale Abnahme des Gehalts an $^{125}$I gemessen und die Veränderungen in der Gesamtpulsfrequenzrate von $^{125}$I markiertem Serumalbumin an der Nase registriert.

Grössere Veränderungen in der Isotopen-Abklingquote wurden bei einer Frequenz von 0.5–6.0 Impulsen/sek beobachtet, während ähnliche Veränderungen des lokalen Blutvolumens und Blutgehaltes bei 0.1–0.5 Impulsen/sek auftraten. Diese Befunde geben Hinweise auf die funktionelle Differenzierung der Vasokonstriktorkontrollen von Austausch- und Speichergefässen unter niedrigem sympathischem Tonus.

Die beobachteten Reaktionen erwiesen sich als Folge einer Aktivierung von sympathischen adrenergen Nerven und Rezeptoren vom α-Typus. Für einen vasodilatatorischen Einfluss durch Stimulierung von β-Rezeptoren oder sympathischen cholinergen vasodilatatorischen Fasern ergaben sich keine Hinweise.

## REFERENCES

Bolme, P. & Edwall, L. 1970. The disappearance of $Xe^{133}$ and $I^{125}$ from skeletal muscle of the anesthetized dog during sympathetic cholinergic vasodilatation. *Acta Physiol Scand 78*, 28.

Dahlström, A. & Fuxe, K. 1965. The adrenergic innervation of the nasal mucosa of certain mammals. *Acta Otolaryng* (Stockh.) 59, 65.

Dawes, J. D. K. & Prichard, M. M. L. 1953. Studies of the vascular arrangements of the nose. *J Anat* (Lond.) *87*, 311.

Drettner, B. 1963. Blood vessel reactions in the nasal mucosa. *Int Rhinology 1*, 40.

Edwall, L. 1971. Some effects of sympathetic nerve activation in oral tissues as studied by tracer disappearance. *Thesis*, Stockholm.

Edwall, L. & Kindlová, M. 1971. The effect of sympathetic nerve stimulation on the rate of disappearance of tracers from various oral tissues. *Acta Odont Scand 29*, 387.

Folkow, B. 1952. *Impulse frequency* in sympathetic vasomotor fibers correlated to the release and

elimination of the transmitter *Acta Physiol Scand 25*, 49
Folkow, B & Hamberger, C-A 1956 Characteristics of sympathetic neuroeffectors in man *J Appl Physiol 9*, 268
Franke, F E 1966 Sympathetic control of the dog's nasal blood vessels *Proc Soc Exp Biol Med 123*, 544
Hall, L J & Jackson, R T 1968 Effects of α- and β adrenergic agonists on nasal blood flow *Ann Otol 77*, 120
Ishii, T & Toriyama, M 1972 Acetylcholinesterase activity in the vasomotor and secretory fibers of the nose *Arch Klin Exp Ohr Nas Kehlkopfheilk 201*, 1
Jodal, M & Lundgren, O 1970 Regional distribution of red cells, plasma and blood volume in the intestinal wall of the cat *Acta Physiol Scand 80*, 533
Kety, S S 1949 Measurement of regional circulation by the local clearance of radioactive sodium *Amer Heart J 38*, 321
— 1960 Theory of blood tissue exchange and its application to measurement of blood flow *Meth Med Res 8*, 223
Malcomson, K G 1959 The vasomotor activities of the nasal mucous membrane *J Laryng 73*, 73
Mellander, S 1960 Comparative studies on the adrenergic neurohumoral control of resistance and capacitance blood vessels in the cat *Acta Physiol Scand 50*, Suppl *176*
Mellander, S & Johansson, B 1968 Control of resistance, exchange and capacitance functions in the peripheral circulation *Pharmacol Rev 20*, 117
Odeblad, E, Westin, B & Englund, S E 1959 Disappearance measurements—theoretical, technical, biological and medical aspects *Acta Radiol* (Stockh ), Suppl *173*
Richerson, H B & Seebohm, P M 1968 Nasal air way response to exercise *J Allergy 41*, 269
Rooker, D W & Jackson, R T 1969 The effects of certain drugs, cervical sympathetic stimulation and section on nasal patency *Ann Otol 78*, 403
Sejrsen, P 1971 Measurement of cutaneous blood flow by freely diffusible radioactive isotopes *Thesis*, Copenhagen
Tschalussow, M A 1913 Die Innervation der Gefasse der Nasenschleimhaut *Pflugers Arch Ges Physiol 151*, 523

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# AN ELECTROMYOGRAPHIC STUDY OF THE VOCAL AND CRICOTHYROID MUSCLES IN FUNCTIONAL DYSPHONIA

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*Abstract* Eighteen patients with dysphonia without any signs of organic lesions as determined by phoniatric and general physical examination and consequently diagnosed as having a functional dysphonia were subjected to EMG examination of the vocal and cricothyroid muscles In all cases indirect laryngoscopy showed the vocal cords to be symmetrical and to have a normal appearance and mobility during quiet breathing During phonation there was an incomplete closure in the posterior part of the vocal cords in 4 patients a minor oval shaped adduction insufficiency ("internus paresis") in 5 patients and in the remaining 9 patients the vocal cords completely closed the rima glottidis Motor unit action potentials were sampled from each investigated muscle by means of a transcutaneous approach and an analysis made by comparison with the muscle action potentials of healthy vocal and cricothyroid muscles The EMG study gave conclusive evidence of neurogenic lesions in 10 cases whereas in 8 patients there were no significant deviations from normal EMG pattern In patients with signs of neurogenic lesions EMG indicated partial paresis in most investigated muscles In two muscles an almost complete conduction block was found Three of the patients had an upper respiratory tract infection preceding their dysphonia a history frequently found in idiopathic vocal cord paresis

Among patients treated at phoniatric departments, 30–40% (Perelló 1962 Brodnitz, 1963) suffer from dysphonia in which organic lesions are not suspected to be a primary cause These patients are commonly said to have a functional disturbance (cf Berendes, 1956 Luchsinger, 1970) The vocal cords appear normal at laryngoscopy, i e there are no apparent changes in the vocal mucosa or the underlying tissue and the range and speed of the movements of the vocal cords are normal In some cases there may be an adduction insufficiency either leaving an oval shaped minor gap between the vocal cords, or resulting in an imperfect closure of the posterior part of the glottis (Beck & Schonharl, 1959, Perelló 1962, Luchsinger, 1970)

The cause of functional dysphonia has been suggested to be a disturbance of the complex co-ordination of the different laryngeal muscles, either of habitual or psychogenic origin (Perelló, 1962) This opinion gains some support from a study of the pattern of the electromyographic activity in the vocal muscles in patients suffering from dysphonia of psychogenic origin Thus, the activity pattern during breathing and phonation was characterised by irregularities in onset, duration and maximal amplitude (Šram & Kalvodová 1965) deviating from that seen in healthy subjects (Faaborg Andersen, 1957) Although deviations in activity pattern were observed, the action potentials did not differ in shape or amplitude from those in healthy vocal muscles, and consequently there was no support for the presence of any peripheral lesion in the nerves or muscles

At the time when these early studies were made, the possibilities of disclosing a partial paresis in a laryngeal muscle were severely

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limited by the lack of sufficient data on the normal distributions of shapes, durations and amplitudes of the motor unit potentials in these muscles Furthermore, the difficulties encountered in identifying a large number of potentials from a single muscle due to the continuous activity with respiratory rhythms (Weddell et al, 1944) render difficult the discrimination of a possible incidence of pathologic action potentials By the accumulation of data on motor unit potentials in healthy vocal (Knutsson et al, 1969) and cricothyroid (Haglund, 1973) muscles from recordings by a method allowing the identification of some twenty motor unit potentials in each muscle, the possibilities of discerning even partial paresis are relatively good This is shown in studies of traumatic (Haglund et al, 1970) and idiopathic (Haglund et al, 1972, 1973 *a*) vocal cord pareses Hence, this method was used in the present study to ascertain whether or not there may be any peripheral injuries in the vocal or cricothyroid muscles in patients with dysphonia where ordinary clinical examinations disclose no relevant signs of organic lesions As will be shown, neurogenic lesions are actually frequent in such cases of dysphonia Consequently, they are not to be regarded as functional and may in several cases be partial idiopathic pareses of muscles innervated by either or both branches of the vagus with motor fibres to the larynx

## MATERIAL AND METHOD

Eighteen patients with dysphonia were studied in which there was no indication of organic lesion as determined by phoniatric and general physical examinations The patients included in the study were 8 men and 10 women with a mean age of 45 years (age range 17–66 years) In all cases the vocal cords were symmetrical and of normal appearance and mobility during quiet breathing as judged from indirect laryngoscopy At phonation there was an incomplete closure in the posterior part of the vocal cords in a few patients, a minor oval shaped adduction insufficiency ( internus paresis") in some others, and in the remainder the vocal cords completely closed the rima glottidis This difference in appearance of the vocal cords at phonation was used to classify the investigated patients into three groups Thus, *group I* consisted of 4 patients with incomplete closure of the posterior part of the glottis This group included 1 man and 3 women (age range 41–48 years) who had suffered from hoarseness from 0 5–30 years *Group II* consisted of 5 patients, 3 men and 2 women with internus paresis" of the vocal cords Here the age range was 49–66 years and the voice disorder was of a 4–10 year duration *In group III* there were 9 patients, 5 men and 4 women (age range 17–62 years) with complete closure of the glottis at phonation Their dysphonia had been present from 1–20 years

In each case an abnormal fatigue and increasing dysphonia was experienced when the voice was stressed by much talking but the degree of the voice disturbance showed great individual differences The aggravations of symptoms disappeared with rest, and the remaining disturbances were of almost constant intensity in all cases except in 4 patients belonging to group III, who at varying intervals had repeated exacerbations of more severe dysphonia for periods varying from a few weeks to 2 months

The patients were subjected to electromyographic examination of the vocal and cricothyroid muscles The exploration was performed by a transcutaneous approach during local anesthesia of the skin and of the subglottic mucosa The procedure of recording from the vocal muscle has been described by Knutsson et al (1969), and that from the cricothyroid muscle by Haglund (1973) Attempts were made to record from each investigated muscle about 20 motor unit action potentials set up spontaneously during quiet breathing The recorded potentials were displayed on an oscilloscope and photographed for later analy shape, duration and amp The a

Table I *EMG data from 4 patients with dysphonia in group I including cases with posterior adduction insufficiency at phonation*

[illegible]

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | Duration of potentials mean ± S D | High amplitude potentials | Polyphasic potentials | Giant potentials | Interference | |
| | | | *n* | (ms) | (%) | (%) | *n* | Pattern | Max amp (mV) |
| Normal m vocalis[b] | | | 469 | 3 76 ± 1 01 | 10 | 0 15 | 0 | Complete | 1 5–2 5 |
| Normal m cricothyreoideus[c] | | | 338 | 3 99 ± 1 40 | 10 | 0–4 | 0 | Complete | 1 2 2 0 |
| *Case* | | | | | | | | | |
| 1 | M vocalis | Right | 39 | 4 01 ± 1 83 | 57** | 21* | 5 | Incomplete | 1 8 |
| | | Left | 25 | 3 22 ± 1 11* | 52** | 8 | 3 | Incomplete | 0 8 |
| | M crico-thyreoideus | Right | 24 | 4 45 ± 1 77 | 54*** | 8*** | 8 | Incomplete | 1 0 |
| | | Left | 24 | 3 65 ± 0 86 | 29* | 8*** | 0 | Incomplete | 1 0 |
| 2 | M vocalis | Right | 26 | 4 75 ± 1 16*** | 65*** | 35*** | 11 | Complete | 1 6 |
| | | Left | 1[a] | — | — | — | — | None | — |
| | M crico-thyreoideus | Right | 21 | 4 18 ± 1 56 | 19 | 4 | 2 | Complete | 2 0 |
| | | Left | 24 | 4 51 ± 1 95 | 47*** | 21*** | 8 | Incomplete | 1 4 |
| 3 | M vocalis | Left | 18 | 3 03 ± 1 09* | 45* | 17* | 3 | Incomplete | 1 5 |
| | M crico-thyreoideus | Right | 8 | 4 94 ± 3 83 | 50*** | 0 | 2 | Complete | 1 0 |
| | | Left | 13 | 3 61 ± 1 13 | 23 | 0 | 1 | Incomplete | 1 0 |
| 4 | M vocalis | Right | 22 | 5 90 ± 1 39*** | 36 | 27*** | 4 | Incomplete | 1 0 |
| | | Left | 45 | 5 28 ± 1 12*** | 38 | 31*** | 8 | Incomplete | 1 1 |
| | M crico-thyreoideus | Right | 41 | 5 46 ± 1 47*** | 34** | 17*** | 10 | Incomplete | 1 1 |
| | | Left | 44 | 4 94 ± 1 64*** | 45*** | 30*** | 8 | Incomplete | 0 9 |

[a] Duration amplitude and shape of the potential 10 ms, 300 μV ten phases
[b] Knutsson et al 1969
[c] Haglund 1973

during deglutition and phonation was recorded from several points in each muscle and its maximum amplitude measured and interference pattern assessed A comparison was made of the data obtained by this procedure and corresponding data from normal vocal muscle (Knutsson et al 1969) and normal cricothyroid muscle (Haglund 1973) When comparing the mean duration of a sample of potentials from a muscle the incidence of potentials with high amplitude and the incidence of polyphasic potentials a statistical analysis was made using the Student s *t* test Extremely large polyphasic or discrete potentials with amplitudes up to 6 mV and often of long duration 5 13 ms were observed in several muscles and they were classified as giant potentials

Since the normal EMG of the cricothyroid muscle has not been available until recently the cricothyroid muscles in 8 early cases were not examined In the remaining 10 cases attempts to record from the two vocal and the two cricothyroid muscles were made but the recordings from one of the vocal muscles in 5 patients and one of the cricothyroid muscles in 3 patients were discontinued This was due to the inability in some subjects to relax sufficiently for identification in the recordings of individual motor unit potentials or to the wearing off of the anesthesia that impeded further exploration Thus results will be given from EMG examinations of 31 vocal and 17 crico-thyroid muscles in the 18 subjects

## RESULT

### *Group I Patients with posterior adduction insufficiency*

Table I gives data from the electromyographic recordings in the four patients with dysphonia and incomplete closure of the posterior part of rima glottidis during phonation It includes mean values and dispersions of the duration of the action potentials identified, incidence of potentials of high amplitude, incidence of polyphasic potentials and number of giant potentials It also includes data on the pattern and maximal amplitude of the interference activity during deglutition and phonation For comparison corresponding values from healthy muscles are also included in the table The incidence of potentials of short duration used in previous reports to assess the probability of discharges of fibrillation potentials (cf Haglund et al, 1972, 1973 *a*) is not given in the table since there was no increased incidence of short potentials of low amplitude in any of the muscles examined in the present study

In one of the muscles, the left vocal of case 2, only one potential was observed despite careful search for activity with the electrode tip at many different sites within the muscle during repeated deglutitions and phonations that normally activate the vocal muscle The observed potential had 10 phases and highly increased duration (10 ms) The findings indicate an almost complete block of nerve conduction to the muscle

An interference pattern was observed at phonation or deglutition in all the other muscles recorded from However, in the majority of muscles the pattern was incomplete and the maximal amplitude of the interference activity somewhat lower than in normal muscles Only in three was the interference pattern judged as complete

The mean duration of the motor unit potentials was within normal limits in 7 of the muscles In 5 muscles it was significantly increased, and in 2 muscles it was somewhat lower than in normal muscles The increase in mean duration was due to an abnormal incidence of potentials of long duration among potentials of normal length so that the dispersions became larger Contrary to previous observations (Haglund et al, 1972, 1973 *a*) the short mean durations in two of the muscles of Table I cannot be ascribed to discharges of fibrillation potentials The short potentials observed were of much larger amplitude than fibrillation potentials Consequently, the low mean duration lacks diagnostic significance in these cases

As in paretic vocal muscles there was a large proportion of potentials of high amplitude in the majority of the muscles in Table I In large samples of potentials collected from a series of normal muscles only 10% had an amplitude exceeding 0 7 mV in the vocal muscle and 0 8 mV in the cricothyroid In samples of 20 potentials from individual normal muscles there were great variations in the incidence of such large potentials Calculations from previous recordings show that the incidence of potentials $\geq 0.7$ mV in 19 normal vocal muscles amounts to $10 \pm 14$% (mean and S D ) The mean incidence of potentials $\geq 0.8$ mV in 17 cricothyroid muscles was $9 \pm 9$% Statistical analysis with Students *t* test showed that the incidence was significantly larger in 10 of the muscles studied in this investigation with the levels of probability indicated in the table Giant potentials were present in all except one of the muscles

The incidence of polyphasic potentials that in healthy vocal muscles is $4 \pm 6$% (mean and S D ) and in healthy cricothyroid muscles is $1 \pm 2$% was significantly increased in 10 muscles

The deviations with respect to mean duration, incidence of high and polyphasic potentials as well as the presence of giant potentials in the muscles of Table I gives strong support for assuming neurogenic lesions in all the patients In the right cricothyroid muscle of case 2 the observation of two typical giant potentials was the only deviation from normal In all the other muscles more than one si

Table I *EMG data from 4 patients with dysphonia in group I including cases with posterior adduction insufficiency at phonation*

The [illegible]

| | | | n | Duration of potentials mean ± S D (ms) | High amplitude potentials (%) | Poly-phasic potentials (%) | Giant potentials n | Interference | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | Pattern | Max amp (mV) |
| Normal m vocalis[b] | | | 469 | 3 76 ± 1 01 | 10 | 0 15 | 0 | Complete | 1 5-2.5 |
| Normal m cricothyreoideus[c] | | | 338 | 3 99 ± 1 40 | 10 | 0-4 | 0 | Complete | 1 2 2.0 |
| *Case* | | | | | | | | | |
| 1 | M vocalis | Right | 39 | 4 01 ± 1 83 | 57** | 21* | 5 | Incomplete | 1 8 |
| | | Left | 25 | 3.22 ± 1 11* | 52** | 8 | 3 | Incomplete | 0 8 |
| | M crico-thyreoideus | Right | 24 | 4 45 ± 1 77 | 54*** | 8*** | 8 | Incomplete | 1 0 |
| | | Left | 24 | 3 65 ± 0 86 | 29* | 8*** | 0 | Incomplete | 1 0 |
| 2 | M vocalis | Right | 26 | 4 75 ± 1 16*** | 65*** | 35*** | 11 | Complete | 1 6 |
| | | Left | 1[a] | — | — | — | — | None | — |
| | M crico-thyreoideus | Right | 21 | 4 18 ± 1 56 | 19 | 4 | 2 | Complete | 2.0 |
| | | Left | 24 | 4 51 ± 1 95 | 42** | 21*** | 8 | Incomplete | 1 4 |
| 3 | M vocalis | Left | 18 | 3 03 ± 1 09* | 45* | 17* | 3 | Incomplete | 1 5 |
| | M crico-thyreoideus | Right | 8 | 4 94 ± 3 83 | 50*** | 0 | 2 | Complete | 1 0 |
| | | Left | 13 | 3 61 ± 1 13 | 23 | 0 | 1 | Incomplete | 1 0 |
| 4 | M vocalis | Right | 22 | 5 90 ± 1 39*** | 36 | 27*** | 4 | Incomplete | 1 0 |
| | | Left | 45 | 5 28 ± 1 12*** | 38 | 31*** | 8 | Incomplete | 1 1 |
| | M crico-thyreoideus | Right | 41 | 5 46 ± 1 47*** | 34** | 17*** | 10 | Incomplete | 1 1 |
| | | Left | 44 | 4 94 ± 1 64*** | 45*** | 30*** | 8 | Incomplete | 0 9 |

[a] Duration amplitude and shape of the potential 10 ms, 300 μV, ten phases
[b] Knutson et al 1969
[c] Haglund 1973

during deglutition and phonation was recorded from several points in each muscle, and its maximum amplitude measured and interference pattern assessed A comparison was made of the data obtained by this procedure and corresponding data from normal vocal muscle (Knutsson et al 1969) and normal cricothyroid muscle (Haglund, 1973) When comparing the mean duration of a sample of potentials from a muscle, the incidence of potentials with high amplitude and the incidence of polyphasic potentials, a statistical analysis was made using the Student's *t* test Extremely large polyphasic or discrete potentials with amplitudes up to 6 mV and often of long duration, 5–13 ms, were observed in several muscles and they were classified as giant potentials

Since the normal EMG of the cricothyroid muscle has not been available until recently, the cricothyroid muscles in 8 early cases were not examined In the remaining 10 cases attempts to record from the two vocal and the two cricothyroid muscles were made, but the recordings from one of the vocal muscles in 5 patients and one of the cricothyroid muscles in 3 patients were discontinued This was due to the inability in some subjects to relax sufficiently for identification in the recordings of individual motor unit potentials or to the wearing off of the anesthesia that impeded further exploration Thus, results will be given from EMG examinations of 31 vocal and 17 cricothyroid muscles in the 18 subjects

only from one vocal cord in each case Complete interference pattern of normal amplitude was observed in the contralateral vocal muscle in both patients but this information is not given in the table

In case 8 in the recordings from the right cricothyroid and vocal muscle there were found significantly increased incidences of potentials of high amplitude Among these there were typical giant potentials Also, two giant potentials were found in the left vocal muscle In the right vocal muscle there was an increased mean duration of potentials In none of the muscles was the interference pattern complete In trials to activate the left cricothyroid muscle by means of repeated deglutitions and phonations, only one action potential of normal appearance was seen other than the noise recorded from distant muscles although the muscle was explored at many points The sensitivity of the fibres to mechanical stimuli appeared to be raised since insertion activity was prominent The findings indicate conduction block in the left cricothyroid muscle and chronic neurogenic lesions in the other three muscles

In case 9 there was an increased incidence of high amplitude potentials in both vocal muscles and giant potentials were recorded from all three investigated muscles A significantly increased mean duration of potentials was found in the left vocal and in the right cricothyroid muscle In all three muscles the interference pattern was incomplete Thus, in all the examined muscles in case 9, signs of a chronic neurogenic lesion were present

In one of the patients of group II, the dysphonia was preceded by an upper respiratory tract infection and developed very suddenly within a matter of hours In the other patients the dysphonia developed without any concomitant symptoms Fatigue after talking and hoarseness were the main symptoms and they had been of relatively constant severity in all cases after the onset of the disturbances which at the time of the EMG examinations had been present for 4–10 years

*Group III Patients with complete closure of the glottis*

Table III shows electromyographic findings in the 9 patients (cases 10–18) with dysphonia in whom the vocal cords were completely closed during phonations In this group of patients the interference pattern during deglutition and phonation was complete in all the muscles examined in 6 of the patients and an incomplete interference pattern being observed in only three of the subjects

In 2 patients (cases 15 and 16) the mean duration of the motor unit potentials in the studied muscles were within normal limits In the right vocal muscle of case 15 there was an almost significant increase of the incidence of polyphasic potentials, but since no other deviations were observed this fact lacks diagnostic validity

In three other subjects (cases 10, 11 and 14) the mean durations in one of the vocal muscles in each case were somewhat lower than in healthy vocal muscles ($P<0.01$) In all these vocal muscles, the short potentials observed had varying amplitudes within the range characteristic for motor unit potentials in normal vocal muscles Consequently, the low mean durations was not due to presence of fibrillation potentials among the action potentials recorded In these subjects an almost significant increase in the incidence of polyphasic potentials was observed in two muscles but neither this nor the low mean durations give any conclusive support for peripheral affections in the muscles that may explain the voice disturbances

In the remaining 4 patients (case 12, 13, 17 and 18), the EMG findings indicated neurogenic lesions Thus in case 12 in whom recordings were obtained from only one vocal muscle, the mean duration and dispersion of durations of the motor unit potentials were increased The incidence of polyphasic potentials was significantly raised and among these polyphasic potentials several were of long duration or high amplitude, one having the

Table III *EMG data from 9 patients with dysphonia in group III including cases with complete clo sure of the vocal cords at phonation*

Data given as in Table I

| | | | n | Duration of potentials mean ± S D (ms) | High amplitude potentials (%) | Poly-phasic potentials (%) | Giant potentials n | Interference Pattern | Interference Max amp (mV) |
|---|---|---|---|---|---|---|---|---|---|
| Normal m vocalis | | | 469 | 3 76 ± 1 01 | 10 | 0–15 | 0 | Complete | 1 5 2.5 |
| Normal m cricothyreoideus | | | 338 | 3 99 ± 1 40 | 10 | 0–4 | 0 | Complete | 1 2 2 0 |
| *Case* | | | | | | | | | |
| 10 | M vocalis | Right | 5 | 3 18 ± 0 60 | 0 | 20* | 0 | Complete | 2 0 |
| | | Left | 25 | 2 93 ± 1 15** | 24 | 16 | 0 | Complete | 2 0 |
| 11 | M vocalis | Right | 18 | 2 96 ± 1 59** | 22 | 17* | 0 | Complete | 1 6 |
| | | Left | 7 | 3 32 ± 1 26 | 0 | 0 | 0 | Complete | 2.0 |
| 12 | M vocalis | Right | 18 | 4 42 ± 1 36* | 22 | 45*** | 1 | Complete | 2 0 |
| 13 | M vocalis | Right | 16 | 3 86 ± 1 03 | 5 | 0 | 0 | Complete | 1 0 |
| | | Left | 19 | 3 84 ± 1.26 | 41* | 0 | 0 | Complete | 1 1 |
| | M crico-thyreoideus | Right | 20 | 4 85 ± 1 57* | 45*** | 30*** | 6 | Incomplete | 1 0 |
| | | Left | 26 | 3 84 ± 1 61 | 50*** | 19*** | 8 | Incomplete | 1.2 |
| 14 | M vocalis | Right | 19 | 3 00 ± 0 81** | 5 | 5 | 0 | Complete | 1 4 |
| | | Left | 9 | 3 18 ± 0 91 | 0 | 10 | 0 | Complete | 1 5 |
| 15 | M vocalis | Right | 24 | 3 65 ± 1 09 | 4 | 21* | 0 | Complete | 1 6 |
| | | Left | 35 | 3 79 ± 1 17 | 3 | 3 | 0 | Complete | 2.5 |
| 16 | M vocalis | Left | 20 | 3 64 ± 0 88 | 25 | 10 | 0 | Complete | 0 8 |
| 17 | M vocalis | Right | 15 | 3 68 ± 1 91 | 13 | 33*** | 2 | Complete | 0 2 |
| | | Left | 21 | 4 68 ± 2.68** | 33 | 33*** | 3 | Complete | 0 8 |
| | M crico-thyreoideus | Left | 9 | 6 38 ± 3 34** | 45*** | 0 | 4 | Incomplete | 1 2 |
| 18 | M vocalis | Right | 49 | 3 92 ± 2 07 | 45* | 25** | 8 | Complete | 1 6 |
| | | Left | 20 | 4 49 ± 1.28* | 55** | 15 | 1 | Complete | 2 4 |
| | M crico-thyreoideus | Right | 16 | 5 16 ± 1 72* | 0 | 6** | 0 | Incomplete | 1 0 |
| | | Left | 20 | 5 41 ± 1 80*** | 30* | 15*** | 4 | Incomplete | 1 6 |

characteristics of a typical polyphasic giant potential

In case 13, the EMG from the two vocal muscles was normal the almost significant increase of potentials of amplitudes > 0 7 mV in one vocal muscle and the somewhat low maximal amplitudes of the interference pattern in the two muscles may well be due to random factors From the two cricothyroid muscles, on the contrary several findings strongly indicate chronic neurogenic lesions in these muscles Thus, means and/or dispersions of the durations of action potentials were increased, and there was an increased incidence of potentials of large amplitude of polyphasic potentials and of giant potentials that were highly significant The electromyographic findings in this patient has been described recently in a case report (Haglund et al, 1973 b)

In cases 17 and 18 the electromyographic activity was quite similar in the two patients An incomplete interference pattern was seen in the three cricothyroid muscles examined and in these the mean durations of the motor unit potentials were significantly increased due to increased proportions of long potentials that were of high amplitude and/or had more than four phases Also in the vocal muscles in these patients, the incidence of long potentials was increased as seen by the increased mean durations in two of the vocal muscles and by the large standard deviations in the two others Polyphasicity was prominent in three of the

vocal muscles and in one of the patients the incidence of high voltage motor units was also significantly raised Giant potentials of extremely high amplitude, some with complex wave forms and long durations were recorded from both vocal and cricothyroid muscles in both cases The findings give conclusive support for assuming chronic neurogenic lesions involving muscles innervated by both the superior and the recurrent laryngeal nerves in these 2 patients

The dysphonias had relatively constant intensities in 5 of the patients in this group (cases 10–14) In the others (cases 15–18) the voice disturbances had intermittent courses with periods of more severe symptoms that lasted for some weeks or months Any infections in connection with the onset of the dysphonia were not recorded, neither were any other concomitant symptoms with the exception of a congenital abnormality in one patient (case 13) She was born with a cleft lip and her voice had been judged as dysphonic from the very start of her talking In addition to fatigue after talking and hoarseness, present in all the patients, her dysphonia was characterised by an inability to raise the pitch of her voice

## DISCUSSION

The results of the present study indicate that neurogenic lesions in laryngeal muscles may be frequent in dysphonia diagnosed as functional from thorough phoniatric and general physical examination In group I, including patients with a posterior adduction insufficiency of the vocal cords, signs of neurogenic lesions were present in all four cases Among the patients with 'internus paresis', group II the larynx EMG was normal in 3 cases and showed signs of neurogenic lesions in 2 cases In group III, including 9 patients with complete closure of the vocal cords, no significant deviations in the EMG were observed in 5 patients and conclusive signs of neurogenic lesions were found in 4 of the patients

In the patients with posterior adduction insufficiency the vocal and cricothyroid muscles on both sides were examined and signs of neurogenic lesions were found in all four muscles of each patient In group II and III all these four muscles were examined only in three of the patients Signs of neurogenic lesions were found in all four muscles in 2 of the cases but in the third such signs were present only in two of the four examined muscles while in the other two the EMG was normal Thus, in some cases the injury may be restricted to some of the muscles In several of the patients in group II and III without EMG changes, all muscles were not examined and the possibility must be taken into consideration that lesions might have been present in some of the muscles not subjected to examination Consequently, the frequency of nerve injuries in patients with functional dysphonia may be still larger than disclosed in the present study although the findings, of course, do not rule out the possibility that the dysphonia in some patients has a functional origin

Deviations in the pattern of activation during phonation as observed in patients with dysphonia (Šram & Kalvodova, 1965) may be caused by disturbances that do not have any easily defined organic origin and thus may be regarded as functional Such deviations might, however, also be due to lesions of the laryngeal nerves, since the demands of recruiting motor units in a muscle subjected to partial paresis will be profoundly altered

In the muscles with neurogenic lesions normal motor unit potentials were found and in many of these muscles the interference pattern was either complete or its maximal amplitude of the same magnitude as in normal muscles The paresis was thus only partial in these muscles This might explain why the position and the mobility of the vocal cords in these patients appear normal or only slightly abnormal However, in the left vocal muscle in case 2 and in the left cricothyroid muscle of case 8 there was evidence of an almost complete conduction block In spite of this, the position and the mobility of the vocal cords

Table III *EMG data from 9 patients with dysphonia in group III including cases with complete closure of the vocal cords at phonation*

Data given as in Table I

| | | | n | Duration of potentials mean ± S D (ms) | High amplitude potentials (%) | Polyphasic potentials (%) | Giant potentials n | Interference Pattern | Interference Max amp (mV) |
|---|---|---|---|---|---|---|---|---|---|
| Normal m vocalis | | | 469 | 3 76±1 01 | 10 | 0–15 | 0 | Complete | 1 5 2.5 |
| Normal m cricothyreoideus | | | 338 | 3 99±1 40 | 10 | 0–4 | 0 | Complete | 1 2 2.0 |
| *Case* | | | | | | | | | |
| 10 | M vocalis | Right | 5 | 3 18±0 60 | 0 | 20* | 0 | Complete | 2 0 |
| | | Left | 25 | 2.93±1 15** | 24 | 16 | 0 | Complete | 2 0 |
| 11 | M vocalis | Right | 18 | 2.96±1 59** | 22 | 17* | 0 | Complete | 1 6 |
| | | Left | 7 | 3 32±1 26 | 0 | 0 | 0 | Complete | 2.0 |
| 12 | M vocalis | Right | 18 | 4 42±1 36* | 22 | 45*** | 1 | Complete | 2.0 |
| 13 | M vocalis | Right | 16 | 3 86±1 03 | 5 | 0 | 0 | Complete | 1 0 |
| | | Left | 19 | 3 84±1 26 | 41* | 0 | 0 | Complete | 1 1 |
| | M crico-thyreoideus | Right | 20 | 4 85±1 57* | 45*** | 30*** | 6 | Incomplete | 1 0 |
| | | Left | 26 | 3 84±1 61 | 50*** | 19*** | 8 | Incomplete | 1 2 |
| 14 | M vocalis | Right | 19 | 3 00±0 81** | 5 | 5 | 0 | Complete | 1 4 |
| | | Left | 9 | 3 18±0 91 | 0 | 10 | 0 | Complete | 1 5 |
| 15 | M vocalis | Right | 24 | 3 65±1 09 | 4 | 21* | 0 | Complete | 1 6 |
| | | Left | 35 | 3 79±1 17 | 3 | 3 | 0 | Complete | 2.5 |
| 16 | M vocalis | Left | 20 | 3 64±0 88 | 25 | 10 | 0 | Complete | 0 8 |
| 17 | M vocalis | Right | 15 | 3 68±1 91 | 13 | 33*** | 2 | Complete | 0 2 |
| | | Left | 21 | 4 68±2 68** | 33 | 33*** | 3 | Complete | 0 8 |
| | M crico-thyreoideus | Left | 9 | 6 38±3 34** | 45*** | 0 | 4 | Incomplete | 1 2 |
| 18 | M vocalis | Right | 49 | 3 92±2 07 | 45* | 25** | 8 | Complete | 1 6 |
| | | Left | 20 | 4 49±1 28* | 55** | 15 | 1 | Complete | 2.4 |
| | M crico-thyreoideus | Right | 16 | 5 16±1 72* | 0 | 6** | 0 | Incomplete | 1 0 |
| | | Left | 20 | 5 41±1 80*** | 30* | 15*** | 4 | Incomplete | 1 6 |

characteristics of a typical polyphasic giant potential

In case 13, the EMG from the two vocal muscles was normal, the almost significant increase of potentials of amplitudes >0 7 mV in one vocal muscle and the somewhat low maximal amplitudes of the interference pattern in the two muscles may well be due to random factors From the two cricothyroid muscles, on the contrary, several findings strongly indicate chronic neurogenic lesions in these muscles Thus, means and/or dispersions of the durations of action potentials were increased, and there was an increased incidence of potentials of large amplitude, of polyphasic potentials and of giant potentials that were highly significant The electromyographic findings in this patient has been described recently in a case report (Haglund et al, 1973 b)

In cases 17 and 18 the electromyographic activity was quite similar in the two patients An incomplete interference pattern was seen in the three cricothyroid muscles examined and in these the mean durations of the motor unit potentials were significantly increased due to increased proportions of long potentials that were of high amplitude and/or had more than four phases Also in the vocal muscles in these patients, the incidence of long potentials was increased as seen by the increased mean durations in two of the vocal muscles and by the large standard deviations in the two others Polyphasicity was prominent in three of the

formte Adduktionsinsuffizienz ("Internusparese") bei 5 Patienten bei den übrigen 9 Patienten kam es zu einem vollständigen Stimmbandschluss der Stimmritze Potentiale motorischer Einheiten wurden von jedem untersuchten Muskel transkutan abgenommen und mit den Aktionspotentialen der gesunden Mm. vocalis und cricothyreoideus verglichen Die EMG Untersuchungen ergaben einen schlussigen Beweis für das Vor' gen einer neurogenen Läsion in 10 Fällen und in 8 Fällen keine oder nur geringfügige Abweichungen vom normalen EMG Muster Bei den Patienten mit Zeichen einer neurogenen Läsion ergab das EMG Hinweise auf eine partielle Parese der meisten untersuchten Muskeln An 2 Muskeln wurde ein nahezu vollständiger Fortleitungsblock gefunden Wie bei den idiopathischen Stimmbandparesen häufig zu beobachten hatten einige Patienten (3) vor dem Auftreten der Dysphonie einen Infekt der oberen Luftwege durchgemacht.

## REFERENCES


Beck J & Schonhärl E. 1959 Die Bedeutung der Stroboskopie für die Diagnose der funktionellen Stimmstörungen *Arch Ohr Nas Kehlkopfheilk 175* 449

Berendes, J 1956 Neuere Ergebnisse über Bewegungsstörungen des Kehlkopfes *Arch Ohr Nas Kehlkopfheilk 169* 1

Brodnitz, F S 1963 Goals, results and limitations of vocal rehabilitation *Arch Otolaryng* (Chic) *77* 148

Faaborg Andersen K. 1957 Electromyographic investigation of intrinsic laryngeal muscles in humans. *Acta Physiol Scand 41* Suppl *140*

Haglund, S 1973 The normal electromyogram in human cricothyroid muscle *Acta Otolaryng* (Stockh) *75* 448

Haglund S Knutsson E Martensson, A & Mårtensson B 1970 Electromyography in vocal cord paresis *Acta Otolaryng* (Stockh) Suppl *263* 85

Haglund, S Knutsson E & Martensson, A 1972 An electromyographic analysis of idiopathic vocal cord paresis *Acta Otolaryng* (Stockh) *74* 265

— 1973 *a*. Neurogenic lesions in the cricothyroid muscle in idiopathic vocal cord paresis *Acta Otolaryng* (Stockh.) *76* 63

— 1973 *b* Electromyography in motor disorders of the larynx. *Acta Otolaryng* (Stockh.) *75* 366

Knutsson, E., Martensson, A & Mårtensson, B 1969 The normal electromyogram in human vocal muscles *Acta Otolaryng* (Stockh) *68* 526

Luchsinger, R 1970 *Handbuch der Stimm und Sprachheilkunde* (ed R Luchsinger & G E. Arnold), Band I, p 430 Springer Verlag Wien and New York

Moody J F 1965 Electrophysiological investigations into the neurological complications of carcinoma. *Brain 88* 1023

Perello J 1962. Dysphonies fonctionelles. *Folia Phoniat 14* 150

Sram, F & Kalvodova, E 1965 Elektromyographische Befunde bei psychogenen Aphonien und spastischen Dysphonien De Therapia vocis et Loquelae Vol II, p 7 Societatis internationalis logopaediae et phoniatriae XIII congressus vindobonae Anno MCMLXV (ed Österreichische Gesellschaft für Logopädie und Phoniatrie Arbeitsgemeinschaft der Sprachheilpädagogen in Österreich, Wiener medizinische Akademie)

Thage O., Trojaborg W & Eu hthal, F 1963 Electromyographic findings in polyneuropathy *Neurology* (Minn.) *13* 273

Trojaborg W., Franzen, E & Andersen I 1969 Peripheral neuropathy and myopathy associated with carcinoma of the lung *Brain 92* 71

Tsairis, P., Dyck P J & Mulder D W 1962 Natural history of brachial plexus neuropathy *Arch Neurol 27* 109

Weddell, G Feinstein, B & Pattle R. E. 1944 The electrical activity of voluntary muscle in man under normal and pathological conditions *Brain 67* 178



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# IMMUNOLOGICAL FUNCTION OF HUMAN TONSILS, WITH SPECIAL REFERENCE TO E- AND EAC-BINDING LYMPHOCYTES

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*Abstract* This investigation was carried out to determine the immunological function of the human tonsil especially the role of lymphocytes The rosette forming technique using sheep red blood cells and complement was used Two types of E and EAC binding lymphocytes were found Whereas the pattern of EAC binding lymphocytes in human tonsil was similar to that in the peripheral blood, the percentage of E binding lymphocytes was less The percentages of rosette forming cells in E or EAC suspensions appeared to be independent of age or sex It was suggested that the human tonsil maintains a lifetime function through an immunocompetent system

It is well known that tonsils play a certain role in the immunocompetent system, although there are inconsistencies among the results of the different authors (Tabata et al, 1967, Surjan et al, 1968, 1972, Godrick & Patt, 1971)

Recently, an advance in immunological techniques (Brain et al, 1970, Lay et al, 1971) demonstrated that lymphocytes from man and primates showed a high proportion of cells adhering to sheep erythrocytes (E), which formed rosettes It is now understood that certain lymphocytes are characterized by membrane markers or receptors for the binding of sheep erythrocytes It has also been confirmed that the lymphocytes binding with E are equivalent to a thymus-dependent or derived cell (T)

Bianco et al (1970) described a population of lymphoid cells from animal species, including man, which were identified through a membrane receptor characteristically bindin with E, sensitized by antibody-complemen These cells are designated EAC-bindin lymphocyte, and their distribution correspond to mouse "B" cells The present investigatio was carried out to determine the following (*a*) the subpopulation of E and EAC bindin lymphocytes in human tonsils, (*b*) a compariso of E- and EAC-binding lymphocytes in peri pheral blood and tonsil, (*c*) effect on the sub population of tonsil cells by age and sex

## MATERIAL AND METHODS

### *Preparation of lymphocyte suspension from peripheral blood and tonsil*

Heparinized peripheral blood and cell suspen sions obtained from minced palatine tonsi were gently placed on the surface of 3 ml o 6% Ficoll with 12% Angio-Conray solution The test tube was then centrifuged at 1 50( rpm for 10 min The lymphocyte rich laye visible in the tube was removed by micro pipette and washed with phosphate bufferec saline (PBS) as shown on Fig 1 This suspen sion then contained 90% of lymphocytes

### *Sheep erythrocytes (E)*

Sheep erythrocytes in Alsever's solution were washed thoroughly in PBS at pH 7 4 and diluted to appropriate suspension of 0 5%

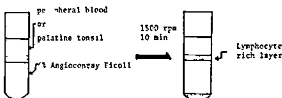





*Fig 1* Schematic drawing and photograph of lymphocyte suspension

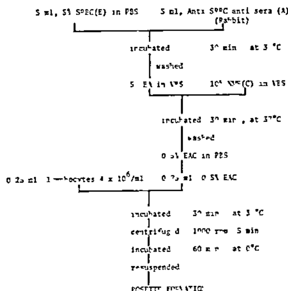


*Fig 3* General procedure of detection of EAC receptor on lymphocytes

*Rabbit anti E anti sera (A)*

The antisera as amboceptor was prepared in rabbit using boiled stroma of E, and 1 250 dilution of antisera was used for EAC reaction

*Detection of receptor in cell suspension*

For detection of E receptor, lymphocyte suspension of 0 25 ml of $4\times10^6$/ml adjusted was mixed with an equal volume of 0 5% E in PBS (pH 7 4) in a test tube and incubated under appropriate condition at 37°C for 5 min The treated mixed cell suspension was gently centrifuged for 5 min at 1 000 rpm and incubated

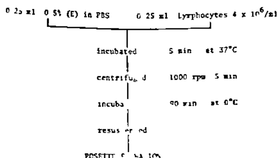



*Fig 2* Detection of E receptor on lymphocytes

at 0°C for 90 min The cells were then resuspended The rosette-forming cells were examined by microscope Detection of EAC receptor was carried out by initially incubating a mixture of 5 ml of 5% E in PBS and 5 ml of anti E sera (A) in rabbit at 37°C for 30 min The sensitized cells (EA) were washed and adjusted to a 5% suspension in Veronal-buffered saline (VBS) at pH 7 4 Equal volume of 5% EA and of normal mouse sera (AKR strain, C) diluted 10% in VBS were mixed The mixture was incubated at 37°C for 30 min and washed gently EAC 0 5% suspension was prepared in PBS at pH 7 4 For the second step, equal volumes of 0 25 ml of 0 5% of EAC and lymphocytes $4\times10^6$/ml were mixed Following incubation at 37°C for 30 min, the mixture was gently centrifuged at 1 000 rpm for 5 min and then incubated at 0°C for 60 min The cell pellet was resuspended and rosette formation was observed under the microscope

The ability to form rosettes with both E and EAC suspension was also tested EAC suspension and lymphocytes were incubated at 37°C for 30 min, then centrifuged Supernatant was withdrawn Cell pellet was resus-

pended and the mixture containing 0 5% E suspension was incubated again at 37°C for 5 min and then centrifuged Final incubation at 0°C for 1 hour was performed and the rosette was examined The percentage of rosette forming cells was determined by microscopic observation of 400 lymphocytes A general outline of the procedure is demonstrated in Figs 1–4

## RESULTS

Microscopic features of rosette forming cells are shown on Fig 5 As is seen in Table I the percentage of lymphocytes from peripheral blood demonstrating rosette forming ability with E suspension was 36 9 by mean values Rosette formation with EAC suspension varied from 25 7 to 47 3% (mean value 40 9%) The ability of cell suspension from palatine tonsil to adhere to E and EAC cells is shown on Table II The percentage of lymphocytes to bind E was 22 6 less than that of peripheral blood whereas EAC suspension represented a mostly similar pattern Similar studies were done to determine the sex or age difference of either E or EAC binding capacity of cell suspension from tonsil

The results are summarized in Tables III and IV Since they demonstrate almost identical patterns it is suggested that the percent

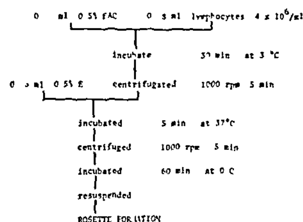



*Fig 4* Detection of both E and EAC receptors on lymphocytes

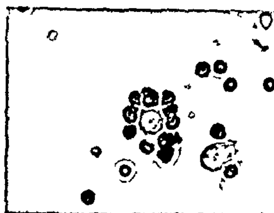


*Fig 5* Microscopic features of rosette forming c of tonsil

ages of rosette forming cells in E or E/ suspension are independent of sex or age

An additional experiment to determine p cisely if the cells forming rosettes were fr an E- or EAC binding cell population reveal that the percentages of binding cells wh lymphocytes were incubated together with bo E and EAC, corresponded mostly to the sum percentages of binding cells to either E or EA (Table V)

## DISCUSSION

Many convincing reports dealing with immun logical problems of rabbit tonsils are four

Table I *Percentages of rosette forming cells peripheral blood with E and EAC suspensions*

| Case | E (%) | EAC (%) |
|---|---|---|
| 1 | 45 2 | 38 4 |
| 2 | 40 6 | 34 4 |
| 3 | 44 7 | 25 7 |
| 4 | 44 4 | 33 8 |
| 5 | 41 0 | 40 8 |
| 6 | 39 5 | 45 0 |
| 7 | 28 3 | 43 5 |
| 8 | 50 0 | 45 9 |
| 9 | 49 5 | 47 3 |
| 10 | 47 5 | 41 8 |
| 11 | 48 2 | 43 7 |
| 12 | 43 0 | 45 2 |
| 13 | 37 2 | 42 7 |
| 14 | 30 8 | 39 0 |
| 15 | 31 6 | 45 3 |
| 16 | 38 4 | 36 1 |
| 17 | 28 6 | 46 2 |
| Mean ± S D | 36 9±6 9 | 40 9±5 6 |

Table II *Percentages of rosette-forming cells of tonsil*

| Case | E (%) | EAC (%) |
|---|---|---|
| 1 | 21 3 | 29 3 |
| 2 | 27 4 | 35 1 |
| 3 | 21 2 | 29 7 |
| 4 | 25 1 | 29 5 |
| 5 | 28 5 | 63.9 |
| 6 | 22 3 | 59 8 |
| 7 | 15 3 | 37 3 |
| 8 | 20 0 | 27.3 |
| 9 | 24 3 | 36 9 |
| 10 | 20 5 | 38 7 |
| Mean±S D | 22 6±3 7 | 38 7±12.2 |

in the literature, yet the real functions are still unknown In our earlier and more recent experiments (Tabata et al, 1967, Okamoto, 1972 *a b*, Tabata et al, 1973), it was proved that the lymphocytes of rabbit tonsil have a mainly ARC-like character on immunological response, and are derived from bone marrow Surjan et al (1972) suggested that rabbit tonsils take part in the immunological process as non regional lymph nodes, playing a role not only as antigen messenger from regional lymph nodes but also in antigen uptake In contrast to the large number of experiments dealing with the immunological function of the rabbit tonsil, there are few reports on human tonsil because of the difficulties of experimental techniques

Recent advance in immunocytology (Bianco et al, 1970 Lay & Nussenzweig, 1968, Lay et al, 1971) demonstrates that there are two lymphocyte populations in peripheral blood

Table III *Sex differences in the rosette forming abilities with E and EAC suspensions*

| Case | Sex | E (%) | EAC (%) | Case | Sex | E (%) | EAC (%) |
|---|---|---|---|---|---|---|---|
| 1 | ♂ | 21 3 | 29 3 | 3 | ♀ | 21 2 | 29 7 |
| 2 | ♂ | 27 4 | 35 2 | 5 | ♀ | 28 5 | 63.9 |
| 4 | ♂ | 25 1 | 29 5 | 8 | ♀ | 20 0 | 27.3 |
| 6 | ♂ | 22.3 | 59 8 | 9 | ♀ | 24 3 | 36 9 |
| 7 | ♂ | 15 3 | 37 3 | 10 | ♀ | 20 5 | 38 7 |
| Mean | | 22 3 | 38.2 | Mean | | 22.9 | 39 3 |

Table IV *Age differences in the percentage of rosette-forming cells with E and EAC suspensions*

| Case | Age | E (%) | EAC (%) |
|---|---|---|---|
| 4 | 11 | 25 1 | 29 5 |
| 5 | 5 | 28 5 | 63 9 |
| 7 | 3 | 15 3 | 37 3 |
| 11 | 3 | 13 5 | 39 5 |
| Mean | | 20 6 | 42 5 |
| 1 | 23 | 21 3 | 29 3 |
| 2 | 37 | 27 4 | 35 2 |
| 3 | 51 | 21.2 | 29 7 |
| 6 | 26 | 22.3 | 59 8 |
| 8 | 20 | 20 0 | 27 3 |
| 9 | 20 | 24 3 | 36 9 |
| 10 | 44 | 20 5 | 38 7 |
| Mean | | 22 4 | 36 7 |

and in lymphoid organ, either of which bind E or EAC

It has also been confirmed that E binding lymphocytes are equivalent to T cell and the others to B cell We were therefore interested to use this technique on the lymphocytes from human tonsil

The present experiment suggests that the human tonsil can supply two different kinds of lymphocytes having immunological properties, such as the thymus or bone marrow derived cells in mice It should be stressed that this cellular pattern of the human tonsil is apparently different from that of rabbit, as previously described by Richter and co-workers (1970) and Tabata et al (1973) It should also be mentioned here that in cells binding with E suspension there is a slight difference in the percentages between peripheral blood and tonsil due to a selective function of post-capillary venules, suggesting the presence of a thymus-dependent area In 1972, Silveira et al re-

Table V *Percentages of rosette forming lymphocytes in tonsils with either or both E and EAC suspensions*

| | E | EAC | E+EAC |
|---|---|---|---|
| Mean (%) | 23 1 | 31 9 | 47 3 |

Table VI *Percentage of lymphoid organ cell suspensions forming rosettes with either E or EAC (Silveira et al, 1972)*

| Suspension tested | Mean % of rosettes | |
|---|---|---|
| | 0 5% E | 0 5% EAC |
| Lymph node | 18 | 21 |
| Thymus | 98 | 0 |
| Spleen | 20 | 31 |
| Thoracic duct | 28 | 41 |

ported that the distribution of the lymphocytes binding with either E or EAC corresponded to thymus-dependent and independent areas in lymphoid organs The percentages of rosette-forming cells with E and EAC suspensions are compared with Silveira's results (Table VI) It is an interesting fact that a pattern of the proportion of the rosette-forming cells in tonsil coincides with that in the thoracic duct Recently, Zucker-Franklin & Berney (1972) selected the surface immunoglobulin bearing cells from human tonsils by reverse immunocyto-adherence technique and pronounced that the distribution of these cells in tonsil was similar to thymus

Absence of sex or age differences in the binding ability of lymphocytes from tonsil with E and EAC cells means that the human tonsil can function throughout life in an immunocompetent system though further investigation on new born specimens should be carried out

## ZUSAMMENFASSUNG

Bei einem Versuch die rosettenbildenden Fahigkeiten der lymphatischen Zellen in den menschlichen Tonsillen zu bestimmen zeigte sich daß es zwei mit *E* und *EAC* gebundene typische Zellen gibt Das prozentuale Verhaltnis der mit *EAC* gebundenen Zellen in den Tonsillen entsprach dem des peripheralen Bluts dagegen zeigten die mit *E* gebundenen Zellen in den Tonsillen eine abweichenden prozentuale Verteilung Weder Geschlecht noch Lebensalter veranderten das prozentuale Verhaltnis der Rosettenbildungsfahigkeit mit *E* und *EAC* Daraus ergibt sich daß die Lymphozyten in den menschlichen Tonsillen zum immunokompetenten System sehr beeinflussen

## REFERENCES

Bianco, C, Patrick, R & Nussenzweig V 1970 A population of lymphocytes bearing a membrane receptor for antigen antibody complement complexes I Separation and characterization *J Exp Med 132* 702

Brain P, Gordon J & Willetts W A 1970 *Rosette* formation by peripheral lymphocytes *Cl n Exp Immun 6* 681

Godrick, E A & Patt, G R 1971 A comparison of the immune response of tonsils with the appendix and spleen *in neonatal rabbits Acta Otolaryng* (Stockh) *71*, 357

Lay W H Mendes N F, Bianco C & Nussenzweig V 1971 Binding of sheep red blood cells to a large population of human lymphocytes *Nature 230* 531

Lay W H & Nussenzweig V 1968 Receptors for complement on leukocytes *J Exp Med 128* 991

Okamoto S 1972 *a* Immunological study on palatine tonsil of rabbit *Acta Otol Jap 64* 983

— 1972 *b* Immunological study on palatine tonsil of rabbit *Acta Otol Jap 65* 795

Richter M Rose B & Abdou N I 1970 Cells involved in the immune response *Int Arch Allergy 38* 269

Silveira N P Mendes N F & Tolnai M E 1972 Tissue localization of two populations of human lymphocytes distinguished by membrane receptors *J Immun 108* 1456

Surján L Surján S & Surjan M 1968 Long term cultivation of lymph nodes fragments and cells of immunized rabbit *Life Science 7* 975

— 1972 Further investigations into the immunological role of tonsils *Acta Otolaryng* (Stockh) *73* 222

Tabata T Okamoto S & Enomoto T 1973 Immunological function of palatine tonsils. I Histological studies of palatine tonsils in thymectomized rabbits In preparation

— 1973 Cell transfer experiments for study of immunological role of palatine tonsils of rabbit *Wakayama Med Rep 15* 211

Tabata T Saito H Sonoda H & Maeda M 1967 Immunological function of palatine tonsils *Wakayama Med Rep 12* 91

Zucker Franklin D & Berney S 1972 Electron microscopic study of surface immunoglobulin bearing human tonsil cells *J Exp Med 135* 533

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# CLINICAL AND ENDOCRINOLOGICAL EFFECTS OF CORIOLIS ACCELERATIONS AND THEIR BEHAVIOR UNDER DRUG TREATMENT

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*Abstract* We have exposed 44 normal human subjects divided into 6 groups, to Coriolis accelerations reproduced in our laboratories The subjects in 3 groups were tested after injection of placebo and those in the other 3 groups after injection of diazepam The clinical and emotional behaviour, nystagmus, urinary 17KS 17OHCS and CA were determined In all subjects exposed to Coriolis accelerations following placebo injection, nystagmus nausea, dizziness and a significant rise in urinary CA were encountered urinary 17KS and 17OHCS were not noticeably modified The subjects tested after intravenous injection of diazepam tolerated the same disturbances with minor emotional reactivity nystagmus, urinary 17KS and 17OHCS were almost unchanged and the urinary output of CA did not show any statistically significant variation Consequently the authors came to the conclusion that for normal subjects exposure to Coriolis accelerations represents a stress which affects the adrenergic but not the hypophysial-cortical system This stress is consistently related to the emotional tension originated by the test itself The possibility of preventing the adrenergic hyperactivity of emotional origin by diazepam is discussed

Coriolis accelerations have reached a remarkable importance in everyday practice of aeronautic and space medicine These accelerations appear in subjects who make head movements during a body rotation at constant angular speed by provoking a flow of endolymph in the semicircular canals, they arouse the excitation of the labyrinth receptors (Schubert 1931 Arslan, 1960, 1961, Bornschein, 1962) A series of clinical disturbances follows, of both subjective (nausea and dizziness) and objective nature (pallor, sweat, vomit and variations in the pulse rate and arterial pressure) these disturbances are called "Coriolis phenomena" (Arslan, 1959, Bornschein & Schubert, 1962)

When Coriolis accelerations occur during acrobatic flights (turns, "tonneau", etc) or space missions, the psycophysical efficiency of pilots and astronauts is restricted (Caporale, 1965) The same troubles have been aroused by head movements in some cosmonauts during their orbital flights (Bergstedt, 1966)

We have examined the relationship between labyrinth excitation and both the adrenergic and the hypophysial-cortical systems in order to reach an objective evaluation of the individual reactivity to the occurrence of Coriolis accelerations At the same time we have studied the possibility of modifying this reactivity by drugs

This research has been made by determining the behaviour of clinical and emotional disturbances, of nystagmus and of urinary catecholamines (CA), 17-ketosteroids (17KS) and 17-Hydroxycorticoids (17OHCS) in groups of subjects affected by Coriolis accelerations after the administration of either placebo or a drug apt to modify the emotional behaviour such as benzodiazepine

## MATERIAL AND METHODS

Studies were performed in 44 normal human subjects (20 men and 14 women) divided into six groups Average age was 29 years (range 17 to 45 years)

In our laboratories Coriolis accelerations have been reproduced as follows the blindfolded subjects were subjected to angular rotation at constant speed whilst sitting on a rotation chair whose rotatory axis passed through the centre of his head, to this rotating motion was associated the additional movements which shifted the head from the original position with respect to the rotatory axis We applied a positive angular acceleration of 0 5°/sec² until we reached a constant angular speed of 90 /sec At this point, we varied the inclination of the chair-back of 45° (backwards) for 3 seconds Three groups were tested after injection of placebo and three groups of an equal number of subjects were tested after injection of benzodiazepine

We have tested various groups of subjects, since the repeated exposure to Coriolis accelerations can diminish or increase the emotional reactivity The nystagmus has been registered by an electronystagmograph incorporated in the rotating chair The urinary 17KS and 17OHCS, collected respectively 4 and 24 hours before and after the experiment, were determined one with the method of Porter & Silber and the other with the method of Collow & Zimmerman Catecholamines (A and NA) were detected in the urine collected 1 hour before and 1 hour after the test, with the method of von Euler (D'Amelio & lon, 1966) Placebo and benzodiazepine[1] were administrated in the brachial vein 15 minutes before the test The placebo consisted of a solution of water and 5 % of glucose

## RESULTS AND DISCUSSION

In all subjects exposed to Coriolis accelerations, after a placebo administration, the Coriolis phenomena have been encountered nystagmus, nausea, dizziness etc The urinary output of 17KS and 17OHCS did not undergo any significant modification Urinary CA was increased in all subjects and the variations were statistically significant It seems evident to us therefore, that in all subjects Coriolis accelerations have stimulated the labyrinth receptors and have represented a stress affecting the adrenergic system but not the hypophysial-cortical system This behaviour is not surprising the stress can act mostly or exclusively on one rather than the other system, as has been reviewed by von Euler already in 1964

The reasons which may have determined a rise in urinary output of CA in subjects tested after i v injection of placebo are various The secretion rate of NA and mostly of A is increased under emotional and nervous strain (Euler, 1964) Besides, an enhanced activity of the sympathetic nervous system can be the counterpart of the enhanced tone of the parasympathetic nervous system (Arslan & Porta, 1947)

The subjects tested after intravenous injection of benzodiazepine, have shown, to a lesser degree, the same Coriolis phenomena, but it is very interesting to remark that they have tolerated the same disturbances with a minor emotional reactivity The nystagmus has appeared almost unchanged, urinary output of 17KS and 17OHCS

Table I

| No of Cases | Urinary catecholamines (nanograms/m') Base | After stimulus | *P* |
|---|---|---|---|
| *Subjects without drug treatment* | | | |
| 7 | 20 40 ± 5 77 | 30 92 − 6 49 | < 0 01 |
| *Subject under drug treatment* | | | |
| 7 | 23 34 ± 6 67 | 29.56 − 7 83 | n s |

Table II

| No of cases | 17 Ketosteroids (urine, mg 4 hours) Base | After stimulus | *P* | 17 Hydroxycorticoids (urine, mg 4 hours) Base | After stimulus | *P* |
|---|---|---|---|---|---|---|
| *Subject without drug treatment* | | | | | | |
| 5 | 2 05 ±0 48 | 2 13 ±0 96 | n s | 1 37 ±0 36 | 1 49 ±0 53 | n s |
| *Subjects under drug treatment* | | | | | | |
| 5 | 3·02 ±0 64 | 2 60 ±0 58 | n s | 2 61 ±0 45 | 2 32 ±0 61 | n s. |

[1] 7-cloro-1,3-didro-1 metil 5 fenil 2H 1 4 benzodiazepine-2-one (Diazepam) at dose of 70 γ/Kg

Table III

| | 17 Ketosteroids (urine, mg/24 hours) | | | 17 Hydroxycorticoids (urine, mg/24 hours) | | |
|---|---|---|---|---|---|---|
| No of cases | Base | After stimulus | *P* | Base | After stimulus | *P* |
| *Subjects without drug treatment* | | | | | | |
| 10 | 11 50 ±2 64 | 10 87 ±4 48 | n s | 8 87 ±2.03 | 9 42 ±2.45 | n s |
| *Subjects under drug treatment* | | | | | | |
| 10 | 8 09 ±3 24 | 9 54 ±4 14 | n s | 10 01 ±1 82 | 9 61 ±1 97 | n s |

(time as above) has not been modified, and urinary output of CA has been enhanced although with lower and not statistically significant variations

One other point remains to be discussed whether the decreased elimination of CA present in all subjects treated with this drug can really be connected with the action of the drug itself This hypothesis is supported by the following considerations Experimental physiological research has proved that diazepam diminishes the excitability of the amygdala and reduces the duration of afterdischarge of the hippocampus, a part of the limbic system which regulates the emotional behaviour (Himwich et al, 1962, Morillo, 1962, Schallek et al, 1964) The activity of the limbic system is strictly connected with the functionality of the hypothalamus, where we find located those sympathetic nervous system centres which activate the processes of integration of the autonomous visceral muscular system in order to mobilize the organic resources in a situation of stress (Arrigo et al, 1965, Himwich et al, 1962)

During some clinical researches, where the individual reactivity to stress has been determined with different parameters such as the modification of the galvanic resistance of the skin and the variation of the respiratory and cardiac frequency it has been proved, in agreement with us that diazepam decreases the reactivity of individuals to stress (Clemens & Selesnick, 1967)

It remains to be established on which of the mechanisms originating a situation of stress after exposure to Coriolis accelerations, the drug can be effective Any interference with the labyrinthine receptors of stimulation may be excluded since the Coriolis phenomena have also appeared in subjects treated pharmacologically, in particular, nystagmus has always been present The clinical observation of lower emotivity, followed by a lower incretion of CA, in subjects treated with diazepam seems rather to prove that this drug—given the functional mechanism described above—acts on the emotional component of stress An investigation on pilots trained for parabolic flights subjects to gravitation is in agreement with this hypothesis In these subjects, exposure to Coriolis accelerations brings on the disturbances originated by labyrinth receptor stimulation but not the hyperincretion of CA (Colehour, 1964) This behaviour is attributed by the author to the elimination of emotional tension and fear through training In the final result the situation is analo gous to that obtained with the administration of diazepam

The experiments of other authors have shown that in the normal subjects emotional stress experimentally induced (film projection) was accompanied by a significantly increased excretion of urinary catecholamines, in three patients with bilateral labyrinthine destruction the adrenalin excretion remained very low throughout the experiment and the emotional stress was not accompanied by the rise found in the healthy subjects These results are very interesting but it is very difficult to compare them with those obtained by us owing to the different experimental conditions (Fluur et al, 1967)

Thus we came to the conclusion that the exposure to Coriolis accelerations represents a stress for a normal subject This stress increases the activity of the adrenergic system but not of the hypophysial-cortical one, and is consistently connected with the emotional tension brought forth by the test itself The possibility of decreasing the adrenergic hyperactivity of emotional origin by drugs (diazepam) can lend itself to useful developments

## ZUSAMMENFASSUNG

44 normale Versuchspersonen, die in 6 Untergruppen unterteilt waren, wurden im rotierenden Stuhl Coriolis-Beschleunigungen ausgesetzt, und zwar wurden diese Teste an den Versuchspersonen von drei dieser Gruppen nach Injektion mit Placebo und an den Versuchspersonen der andern drei Gruppen nach Injektion von Diazepan durchgeführt Das klinische und emotionelle Verhalten, Nystagmus und der Gehalt an 17-Ketosteroiden (17-KS), 17-Hydroxycorticoiden (17-OHCS) und Katecholaminen (CA) im Harn wurden bestimmt.

Bei allen Versuchspersonen, die nach Placebo-Injektion Coriolis-Beschleunigungen ausgesetzt worden waren, wurde Nystagmus, Übelkeit, Schwindelgefühl und ein signifikantes Ansteigen an CA im Harn festgestellt Dagegen zeigten 17-KS und 17-OHCS im Harn keine merkbare Änderung Die Versuchspersonen, die nach intravenöser Injektion von Diazepan getestet wurden, tolerierten die gleichen Störungen mit geringerer emotioneller Reaktivität der Nystagmus und der Gehalt an 17-KS und 17-OHCS im Harn waren fast unverändert und der CA-Betrag im Harn zeigte keine statistisch signifikante Veränderung. Die Autoren kamen daher zu dem Schluss, dass die Belastung mit Coriolis-Beschleunigungen für normale Versuchspersonen einen Spannungszustand (Stress) bedeutet, der das adrenerge, aber nicht das hypophysär-cortikale System beeinflusst Dieser Stress ist engstens verkoppelt mit der emotionellen Anspannung, die durch den Test selbst ausgelöst wird Die Möglichkeit, der adrenergen Hyperaktivität emotionellen Ursprungs durch Diazepan vorzubeugen, wird diskutiert

## REFERENCES

Arrigo, A, Jann, G & Tonali, P 1965 Some aspects of the action of Valium and of Librium on the electrical activity of the rabbit brain *Arch Int Pharmacodyn 54*, 364

Arslan, M 1959 Ricerche sperimentali sui fenomeni vegetativi che insorgono a seguito delle accelerazioni di Coriolis 2 Congr Mond e 4 Europ di Med Aeron e Spaz, Roma

— 1960-61 Le accelerazioni di Coriolis e i recettori labirintici *Sci Matematiche e Naturali 73*

— 1961 Les accélerations de Coriolis dans la stimulation vestibulaire (recherches ENG) *Confin Neurol 21*, 403

Arslan, M & Porta, C F. 1947. Apparato auricolare e sistema nervoso vegetativo Relazione 26° Congr Soc. Ital di Laring Otol Rinol, Genova

Bergstedt, M 1966 Vestibular problems in space travel. *Acta Otolaryng* (Stockh), Suppl *224*

Bornschein, H 1962 Theorie und praktische Bedeutung des vestibularen Coriolis-Effektes *Mschr Ohrenheilk* 96, 45

Bornschein, H & Schubert, G 1962 Die Richtung des vestibularen Coriolis-Effektes *Z Biol 110*, 269

Caporale, R 1965 L'apparato vestibolare nel volo al mosferico e spaziale *Min Med 56*, 2035

Clemens, T. L & Selesnick, S T. 1967 Psychological method for evaluating medication by repeated exposure to a stressor film *Dis Nerv Syst 28*, 98

Colehour, J K. 1964 The effects of Coriolis acceleration during zero-gravity parabolic flight *Aerospace Med* 9, 844

D'Arrelio, G & Celon, E 1956 Eliminazione urinaria della adrenalina e noradrenalina nell ipertensione arteriosa essenziale *Minerva Med 57*, 2623

Euler, v U S 1964 Quantitation of stress by catecholamine analysis *Clin Pharmacol Ther 5*, 398

Fluur, E Gejrot, T & Levi, L 1957 Sympatho-adrenomedullary activity during experimentally provoked mental stress in patients with labyrinthine defects. *Acta Otolaryng* (Stockh) Suppl *224*, 260

Himwich, H E, Morillo, A & Steiner, W G 1962 Drugs effecting rhinencephalic structures *J Neuropsychiat*, Suppl *3*

Marchiori, C 1966 Effetti delle accelerazioni complementari di Coriolis sul nistagmo post-rotario *Boll Soc Ital Biol Sper 42*, 723

Morillo, A 1962 Effects of benzodiazepines upon amygdala and hippocampus of the cat *Int J Neuropharmacol 1*, 353

Schallek, F, Zabransky, F & Kuehn, A 1964 Effects of benzodiazepines on central nervous system of cat *Arch Int Pharmacodyn, 149*, 467

Schubert, G 1931 Über die physiologischen Auswirkungen der Coriolis-Kräfte bei Trudelbewegung des Flugzeuges *Acta Otolaryng* (Stockh) *16*, 39

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# SELECTIVE HABITUATION OF VESTIBULAR NYSTAGMUS BY VISUAL STIMULATION

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*Abstract* The effect on vestibular nystagmus of repeated exposure to a unidirectional rotating visual field was studied in ten subjects A visually induced sensation of self rotation (circularvection – CV) about a vertical axis was repeated 30 times for each subject Post stimulation rotation with eyes closed showed a significant 40% reduction in vestibular nystagmus intensity in the direction of the CV, measured by cumulative displacement, and no significant reduction in the opposite direction

Vestibular habituation, the reduced nystagmus and subjective response upon repeated vestibular stimulation, is a well known phenomenon which can be produced in the laboratory (by rotation or caloric stimulation) and which occurs normally in dancers, skaters, pilots and others experiencing repeated motion patterns The practical protective role of habituation, in preventing the disorientation and high nystagmus level associated with strong semicircular canal stimulation, is evident An extensive review of the many animal and human vestibular habituation studies (Collins, 1973) leads to the interpretation of vestibular habituation as the development of conditioned compensatory reactions to oppose inappropriate responses associated with visual vestibular conflicts and exposure to unusual motion environments

Most of the habituation experiments have shown the habituation to be quite specific to the stimulus situation, as to the direction, plane and frequency of the rotation, the orientation of the head, the visual input and the manner of stimulating the labyrinth Repeated caloric testing, although habituating the nystagmic response to further caloric stimuli, does not generally transfer to reduce the response to rotation (Collins, 1973) Only by repeated equivalent binaural caloric stimulation with visual fixation was any transfer of habituation to a rotation test observed (Pfaltz & Piffko, 1970) There are a number of situations in which non vestibular inputs have been shown to influence vestibular responses In addition to the important influence of arousal on vestibular nystagmus (Torok, 1970), the response is effected by visual fixation and optokinetic stimulation (Jung, 1948), and by a conditioned response using auditory stimulation (Arslan et al, 1970) Acquisition of vestibular habituation can be blocked by anesthesia, and its effectiveness reduced by adrenaline (Hood & Pfaltz, 1954) and by ingestion of alcohol (Aschan et al, 1956) Furthermore, there is indirect evidence of common central mechanisms for generation of optokinetic and vestibular nystagmus (Trincker et al, 1961, Tibbling 1970, Fukuda & Tokita, 1964) An unanswered question was whether or not a pure visual stimulus could reduce the response to a subsequent pure vestibular stimulus, demonstrating

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a transfer of habituation across sensory modalities

Under appropriate visual conditions a large peripheral field, moving uniformly around a subject, is known to produce a sensation of self rotation (circularvection, or CV), which can be extremely powerful and nearly indistinguishable from a true body rotation (Mach 1875, Fischer & Kornmüller, 1930, Dichgans & Brandt, 1972, Young et al, 1973) Although this effect can be produced about horizontal as well as vertical axes (Dichgans et al, 1972) it is most similar to a normal vestibular rotation test sensation for rotation about the vertical Circularvection may be produced either with or without a fixation point Repeated exposure to a small optokinetic stimulus without a fixation point, does not reduce OKN, but rather increases it, nor does it reduce the vestibular response measured by caloric or galvanic stimuli (Miyoshi et al, 1973) Repeated optokinetic stimulation does, however, produce a directional change in the stepping test in men (Miyoshi et al, 1973) and in head nystagmus in animals (Hinoki & Terayama, 1966) We created an experimental situation in which a pattern of moving stripes created a *sensation of rotation* which closely resembled that of the vestibular rotation tests but did not induce OKN because of a fixation point We studied the effect of repeated visual stimuli on the response to a vestibular rotation test administered afterwards

## METHODS

### *Subjects*

Thirteen subjects from the hospital staff were tested, none having known vestibular or neurological disorders The three women and ten men ranged in age from 20 to 47 Three subjects were excused from further testing when the nystagmography revealed spontaneous nystagmus with eyes closed In the remaining group some subjects showed varying degrees of direction preponderance on vestibular testing Three other subjects were given a reversed order pre test and post test to check for the effect of test order The remaining seven subjects underwent the normal protocol described below

### *Pre-test and post-test rotations*

Before and after exposure to the visual stimuli all subjects were accelerated right and left to test the strength of vestibular nystagmus in each direction In the normal protocol, as shown in Fig 1*a*, subjects were accelerated clockwise about a vertical axis, at an acceleration of 5 deg/sec$^2$ for 12 sec, rotated at a constant speed of 60 deg/sec for 45 sec and finally decelerated at 5 deg/sec$^2$ to a stop Motion was controlled using a Tönnies (Freiburg im Breisgau/Germany) rotating chair All tests were performed with eyes closed in the dark Nystagmus and subjective sensation of rotation were recorded from 30 sec prior to acceleration until 30 sec after the chair had come to a stop For the reversed order protocol, the pre and post tests were performed with counterclockwise rotation the direction of the visual stimuli remaining unchanged For one subject (7) all accelerations were for 15 sec, and the constant velocity period was at 75 deg/sec

### *Control tests*

To separate the habituating potential of the visual stimuli from any response decline resulting from the repeated rotation the pre test and post test sequence was also administered to each subject at a separate t[illegible] from the habituation test No intermed[illegible] moving stripe stimulus was given To av[illegible] order effects and habituation to repeated test[illegible] and to investigate the duration of any visu[illegible] induced habituation the control test was gi[illegible] to each subject only once, either in the mo[illegible] before the main experiment or during the th[illegible] months after the experiment

### *Visual stimulus*

The visual stimulus used to generate circular[illegible]tion about a vertical axis was similar to t[illegible] used in other circularvection studies (Dichg[illegible] & Brandt 1972, Young et al, 1973) The T[illegible]nies moving shadow optokinetic stimulus ge[illegible]

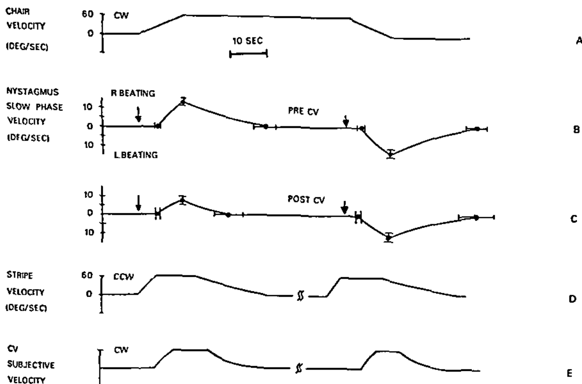



*Fig 1* Average pre- and post test nystagmus responses and typical CV stimuli (*A*) Pre and post test chair angular velocity, normal protocol (*B*) Average pre-test vestibular nystagmus response, ten subjects Circles show mean latency, mean slow phase velocity averaged over ten seconds (see methods), and mean duration Bars show standard error of these means Lines connecting data points are merely suggestive of the nystagmus waveform Arrows line up with onset of acceleration (*C*) Average post CV vestibular nystagmus, as in *B* (*D*) CV stimulus stripe velocity Two representative waveforms of a series of 30 (*E*) Sketch of typical CV velocity estimates corresponding to the stimuli in *D*

rator was modified to generate a desired circularvection dynamic response The subject was centered before a white screen, one meter in radius The screen was 135 cm high and covered a visual angle approximately 31 deg above, 37 deg below, and 80 deg to each side of the eyes in primary position The dark stripes subtended 6°40 and were separated by 8 deg The subject held his head fixed and upright in a dental head rest, and fixated a small (19 mm × 19 mm) dark square placed in the center of the screen at eye level The visual stimulus was a stripe motion in the counterclockwise direction, producing only clockwi subjective velocity For each CV exposure the stripes were accelerated from rest to a speed of 60 deg/sec counterclockwise (75 deg/sec for subject 7) over a period varying from 3 to 15 sec The constant velocity period lasted from 10 to 60 sec, following which the stripe speed slowed exponentially toward zero velocity with a time constant of 12 sec and stopped after 15 to 30 sec The movement pattern of the stripes (acceleration, constant velocity, and deceleration) was chosen to produce corresponding sensations of increasing, constant, and decreasing CV It was intended to produce CV patterns which mimic the counterclockwise sensation produced by the first part of the rotation stimulus used in the pre- and post-tests This procedure was repeated 30 times with pauses of 5–20 sec between trials

*Subject instructions and control of arousal*

Subject arousal was maintained at a high level to promote nystagmus by requiring the signalling of 90 deg turn intervals (Collins & Guedry,

1962, Collins, 1964) During the pre- and post-rotation tests, subjects were instructed to close their eyes, look straight ahead, and concentrate on the signalling task A small three position switch was used to indicate the time and direction of the onset of rotation, each 90 degree interval, and the cessation of turning During the circularvection tests subjects were instructed to look at the fixation point and indicate any self motion in the same manner as during rotation in the dark

*Data recording and analysis*

Horizontal eye movements were recorded by electronystagmography, with a 3 sec time constant Eye movement records were hand analyzed by one of us unaware of the direction of CV for each subject Cumulative eye displacement, used as a measure of average slow phase velocity, was measured over the peak 10 sec nystagmus activity period by taking the sum of all fast phase amplitudes Beat frequency was calculated for this same period Nystagmus latency was measured from the onset of acceleration or deceleration to the first fast phase, and duration was measured to the last fast phase Subjective turning latency, peak subjective velocity and rotation were also calculated from the switch cations

All statistical analyses were performed using T-tests on paired differences

## RESULTS

*In all* subjects tested vestibular nystagmus for acceleration in the direction of circularvection was reduced following exposure to the visual stimulus The average nystagmus response, for all ten subjects is shown in Fig 1*B* for the vestibular pre test and in Fig 1*C* for the post test The average latency duration and peak slow phase velocity (as estimated from cumulative eye displacement) are indicated with the standard error of these averages The right and *left beating nystagmus of the reversed order* protocol subjects were averaged with the normal protocol right and left nystagmus respectively

All subjects experienced CV in the direction opposite to stripe motion Typical stripe velocity wave forms are shown in Fig 1*D*, with sketches of common subjective turning indications given *in Fig 1E The subjective indication of turning* usually commenced 3–10 sec following the beginning of stripe motion, continued at a relatively constant rate during constant speed stripe rotation, and declined gradually during the decay of stripe speed No consistent pattern of change in subjective response latency or peak velocity was seen during the thirty CV trials Because of the fixation point, no measurable optokinetic nystagmus occurred during the CV trials

Fig 2 shows one case of nystagmus decline In this case an initially strong and regular right beating vestibular nystagmus (top trace) was reduced markedly in slow phase velocity, frequency and duration following exposure to thirty CV-right stimuli (second trace) The weaker initial left beating vestibular nystagmus (third trace), on the other hand showed only a slight decline following the CV stimuli (bottom trace)

*Cumulative displacement*

As expected, of the various nystagmus parameters measured, only cumulative displacement showed a clear and consistent direction specific response reduction Table I lists the cumulative displacement for each subject, in each direction, pre- and post-exposure to the CV stimuli, and for the control tests The post test cumulative displacement *declines relative to the pre test* level in the direction of CV, for each of the ten subjects The average decline is 40 % and is significantly different from zero ($p < 0.0025$) In the direction opposite to CV, on the other hand a response decline in cumulative displacement was only present in 7 of the ten subjects (average 16 %) and was not significant at the 0 1 level When each subject's decline is compared as to the direction of acceleration relative to CV, the *declines in the direction of CV* are an average of 23 % larger than those for acceleration opposite to CV ($p < 0.05$) The pre- and post test

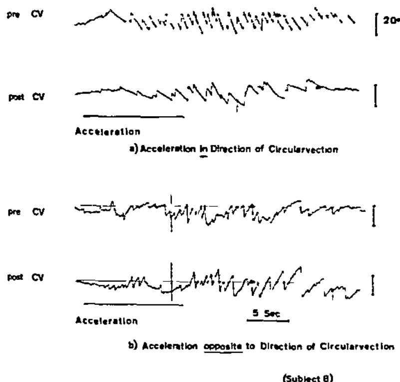



*Fig 2* Vestibular nystagmus recordings from one subject before and after exposure to 30 unidirectional circular vection stimuli

displacements for each subject in the control tests, without any CV, indicate no consistent or significant response decline associated with the repeated testing

When each subject's response decline in the direction of CV is compared with his own change in response in that direction for the control test, this percentage decline is an average of 53 % larger ($p < 0.0025$) Thus one effect of the repeated unidirectional CV trials is to reduce significantly the vestibular nystagmus cumulative displacement for true body acceleration in the direction of CV, relative both to control tests without CV and to accelerations in the opposite direction

### *Other measures*

The only other nystagmus parameter found to be effected by the CV tests was the average frequency of nystagmus during the peak velocity period Contrary to the findings of Johnson & Torok (1970) who measured vestibular nystagmus frequency with eyes open in the dark, and the earlier findings of Wendt (1965) and Collins (1964) nystagmus frequency decreased slightly in the post-tests The average frequency declined 23 % in the CV direction ($p < 0.1$) and 21 % in the direction opposite to CV ($p < 0.1$) There was no significant difference between the two directions The frequency measures were found to be more variable and less sensitive to habituation than the measures of slow phase velocity It is conceded that the measurement of frequency is a less reliable parameter, especially when recording conditions are noisy To exclude such errors only fast phases with amplitudes of more than 2 deg were counted The resolution of the recording system was better than 1 deg Latency and duration measures did not show any significant evidence of any habituation Subjective measures were similarly unrevealing

### *Duration of habituation*

Although a systematic investigation of the duration of the visually induced vestibular nystagmus habituation was not part of the

Table I *Vestibular nystagmus cumulative displacement, in degrees, measured during the peak velocity ten second period for each acceleration*

| | With CV stimuli | | | | Without CV stimuli | | | |
|---|---|---|---|---|---|---|---|---|
| | Acc w CV (clockwise) | | Acc opp CV (counterclockwise) | | Clockwise acc | | Counterclockwise acc | |
| Subject | Pre | Post | Pre | Post | Pre | Post | Pre | Post |
| 1 | 57 | 48 | 37 | 37 | 106 | 103 | 98 | 125 |
| 2 | 138 | 46 | 140 | 202 | 81 | 102 | 125 | 101 |
| 3 | 68 | 45 | 57 | 45 | 170 | 240 | 167 | 207 |
| 4 | 122 | 16 | 97 | 8 | 154 | 132 | 140 | 182 |
| 5 | 137 | 17 | 136 | 36 | 138 | 110 | 137 | 88 |
| 6 | 110 | 85 | 188 | 130 | 86 | 122 | 29 | 51 |
| 7* | 205 | 154 | 200 | 179 | 74 | 112 | 120 | 80 |
| 8 | 168 | 78 | 125 | 122 | 105 | 158 | 133 | 130 |
| 9 | 255 | 250 | 337 | 280 | 230 | 241 | 229 | 194 |
| 10 | 51 | 49 | 56 | 77 | 107 | 67 | 163 | 115 |
| Average decline pre-post tests | 40% ($p$ 0 0025) | | 16% (NS) | | −13% (NS) | | −2% (NS) | |

* – tested at 75 deg sec, data normalized to 60 deg sec

experimental plan, incidental observations are relevant Control rotation tests were performed on seven subjects at times ranging from two weeks to three months following the CV stimulus In no case did any sign of the earlier habituation reappear Furthermore, four of the subjects were given a second acceleration post test, a few minutes after the first post-test, hich immediately followed the CV stimulus ries In each of these re-tests, the habituation (primarily in the direction of the CV as seen in the first post-test) was nearly completely absent in the second test Finally, for two of three subjects (8–10), receiving the reversed order protocol, in which they were tested in the opposite CV direction immediately after the CV series, and then in the CV direction, the reduction in the CV direction was small All of these observations point to the conclusion that the response reduction resulting from this short, one-half hour exposure to a CV stimulus, is extremely brief compared to the long duration response reductions associated with repeated accelerations (Hood & Pfaltz, 1954, Collins, 1964) They also confirm the importance of testing order in searching for vestibular directional effects (Aschan et al, 1952)

## DISCUSSION

Several lines of evidence suggest that vestibular habituation is of central rather than of peripheral origin Some of the more persuasive experiments supporting this view come from the inhibiting effect of the efferent vestibular processes (Groen, 1965, Goetmakers, 1970), the specific habituation to certain patterns of motion (Aschan 1954, Collins, 1968), and the importance of alertness in regulating the level of nystagmus and response reduction (Collins, 1964, Guedry, 1965, Benson *et al*, *1966*) *Of particular interest is the fact* that stimulation of the vestibular organ is not, in itself, sufficient to produce vestibular habituation under slightly altered circumstances Habituation gained with vestibular stimulation in one head orientation does not transfer to an altered head position, to a different axis of rotation, or to a new direction of acceleration about the same axis (Collins, 1969, Crampton & Brown 1964, Collins, 1964) Finally, the failure to obtain transfer of habituation from one stimulus mode to another is particularly noteworthy Thus stimulation of the vestibular apparatus has been shown not to be a *sufficient* condition for *vestibular habituation* The current experiment

demonstrates that it is also not a *necessary* condition, since exposure to a purely non-vestibular input led to vestibular habituation The term "vestibular habituation", as generally used to refer to a decline in the vestibulo-ocular response upon repeated tests is appropriately used for the phenomenon found in this experiment, even though no adequate vestibular stimulus was involved in the habituation process On the other hand, the absence of OKN during the CV trial precludes any localization of the habituation on the motor side The transfer of habituation across sensory modalities is a proof of its central origin

The reason for the success of CV habituation transfer to rotation stimuli and the usual failure of caloric habituation to transfer probably is associated with the different orientation of the head (Collins, 1964, 1969) The similarity of the effects of CV stimulation to those of true rotation extend to the influence on vestibular thresholds (Young et al, 1973) and the production of the pseudo-coriolis sensation (Dichgans & Brandt, 1973) The production of nystagmus by the habituating stimulus seems unrelated to the efficacy of the stimulus in habituating vestibular nystagmus associated with rotation in the dark Furthermore, Brandt et al (1973) have shown the independence of CV and optokinetic nystagmus We cannot, of course, eliminate the possibility that the stimulus is one which would tend to produce nystagmus, but that the visual fixation system counters this and that the process of actively countering nystagmus is, in itself contributory to the habituation

One interpretation of this experiment, in conjunction with the caloric transfer experiments, is that a habituating stimulus must produce the same subjective sensation of motion as that produced by true body motion in order to habituate vestibular nystagmus in the latter case Stimuli which do not produce identical subjective sensations, because of conflicting visual-vestibular relationships or because of intra-labyrinthine conflicts, are not adequate to transfer nystagmus habituation between different modes of stimulation even though they may produce similar nystagmus patterns As Nilsson (1972) has pointed out, vestibular nystagmus and sensation of turning, although responding to the same stimuli and following similar time courses, are only weakly correlated and are regulated at two separate levels

Speculation on the site of the visually induced habituation is somewhat premature The finding of the current experiment, that visual inputs inhibit a vestibular response in a manner entirely similar to that of vestibular inputs, is consistent with the hypothesis that the visual input in fact biases the vestibular signal and produces, at the neural level, a change in firing pattern which is indistinguishable from that produced by pure vestibular stimulation (Klinke & Schmidt, 1970, Dichgans & Brandt, 1972, Dichgans et al, 1973, Henn et al, 1973)

## ZUSAMMENFASSUNG

Der Effekt eines sich nach rechts drehenden visuellen Feldes auf vestibularen Nystagmus wurde bei 10 Versuchspersonen untersucht. 30mal wurde bei jeder Versuchsperson eine visuell induzierte Empfindung von Eigenrotation (Zirkularvektion–CV) um eine vertikale Achse hervorgerufen Danach war der vestibulare Nystagmus bei Drehung mit geschlossenen Augen in Richtung der CV, gemessen als kumulative Augenabweichung, um 40 % signifikant vermindert In der Gegenrichtung war er nicht signifikant reduziert

## REFERENCES

Arslan, M, Megighian, D & Marchiori, C 1970 Evocation of vestibular nystagmus with the technique of conditioned reflexes *Advance Otorhinolaryng 17*, 63

Aschan, G 1954 Response to rotatory stimuli in fighter pilots *Acta Otolaryng* (Stockh) Suppl *116*, 24

Aschan, G, Bergstedt M, Goldberg, L. & Laurell, L, 1956 Positional nystagmus in man during and after alcohol intoxication *Quart J Stud Alcohol 17*, 381

Aschan, G, Nylén, C O, Stahle, J & Wersall, R 1952. The rotation test Cupulometric data from 320 normals *Acta Otolaryng* (Stockh) *42*, 451

Benson A J Goorney, B B & Reason J T 1966 The effect of instructions upon post rotational sensations and nystagmus. *Acta Otolaryng* (Stockh) *62*, 442

Brandt, Th Dichgans, J & Koenig, E 1973 Differential effects of central versus peripheral vision on egocentric and exocentric motion perception. *Exp Brain Res 16*, 451

Collins, W E 1964 Task-control of arousal and the effects of repeated unidirectional angular acceleration

on human vestibular responses *Acta Otolaryng* (Stockh), Suppl *190*, 1
Collins, W E 1968 Special effects of brief periods of visual fixation on nystagmus and sensations of turning *Aerospace Med 39*, 257
— 1969 Influence of head position on the habituation of vertical vestibular nystagmus in the cat *J Comp Physiol Psychol 67*, 156
— 1973 Habituation of vestibular responses an overview In *Handbook of sensory physiology*, vol VI Vestibular system (ed H H Kornhuber) Springer, Berlin (in preparation)
Collins, W. E & Guedry, F E, Jr 1962 Arousal effects and nystagmus during prolonged constant angular acceleration *Acta Otolaryng* (Stockh) *54*, 349
Crampton, G H & Brown J H 1964 Repeated vertical semicircular canal stimulation does not habituate horizontal nystagmus in cat *Acta Otolaryng* (Stockh) *58*, 441
Dichgans J, Schmidt, C L & Graf, W 1973 Visual input improves the speedometer function of the vestibular nuclei in the goldfish Exp Brain Res *18*, 319
Dichgans, J & Brandt, Th 1972 Visual vestibular integration and motion perception In *Cerebral control of eye movements* (ed J Dichgans & E Bizzi), p 327 (Bibl Ophthal *82*) Karger, Basel
— 1973 Optokinetic motion sickness and pseudocoriolis effects induced by moving visual stimuli *Acta Otolaryng* (Stockh) *76*, 339
Dichgans, J, Held, R., Young, L R & Brandt, Th 1972. Moving visual scenes influence the apparent direction of gravity *Science 178*, 1217
Fischer, M H & Kornmuller, A E 1930 Optokinetisch ausgelöste Bewegungswahrnehmungen und optokinetischer Nystagmus *J Psychol Neurol* (Lpz) *41*, 273
Fukuda T & Tokita, T 1964 Diagonal nystagmus Influence of optokinetic stimulation on postrotatory and caloric nystagmus *Confin Neurol 24* 127
Goetmakers R 1970 Vestibular adaptation in Rana *Advance Otorhinolaryng 17*, 107
Groen J T 1965 Central regulation of the vestibular system *Acta Otolaryng* (Stockh) *59* 211
Guedry F E 1965 Psychological studies of vestibular function In *Contributions to sensory physiology* (ed W D Neff) Academic Press New York
Henn V, Young L R, & Fineley, C 1973 Visual input in the nucleus vestibularis of the alert monkey *Pflugers Arch Ges Physiol*, Suppl *343*, 171
Hinoki, M & Terayama, K 1966 Studies on the optokinetic nystagmus from the standpoint of body equilibrium *Acta Otolaryng* (Stockh) *62* 8
Hood, J D & Pfaltz C R 1954 Observations on the effects of repeated stimulation upon rotational and caloric nystagmus *J Physiol 124* 130
Johnson, D D & Torok N 1970 Habituation of nystagmus and sensation of motion after rotation *Acta Otolaryng* (Stockh) *69*, 206
Jung, R 1948 Die Registrierung des postrotatorischen und optokinetischen Nystagmus und die optisch vestibuläre Integration beim Menschen *Acta Otolaryng* (Stockh) *36*, 199
Klinke, R. & Schmidt, C. L. 1970 Efferent influence on the vestibular organ during active movement of the body *Pflugers Arch Ges Physiol 318*, 352
Mach, E 1875 *Grundlinien der Lehre von den Bewegungsempfindungen* Wilhelm Engelmann, Leipzig (also E.S Bonset, Amsterdam, 1967)
Maxwell, S S, Burke, U L & Reston, C 1922 The effect of repeated rotation on the duration of after nystagmus in the rabbit *Amer J Physiol 58*, 432.
Miyoshi, T, Pfaltz, C R & Piffko, P 1973 Effect of repetitive optokinetic stimulation upon optokinetic and vestibular responses *Acta Otolaryng* (Stockh) *75*, 259
Nilsson, A 1972 Comparisons between oculogyral illusion, turning sensation and nystagmus within a process frame of reference In *Visual aftereffects and the individual as an adaptive system* (A Andersson, A Willson, E Ruuth & G Smith) CWK Gleerup Lund, Sweden, 1972.
Pfaltz, C R & Piffko, P 1970 Studies on habituation of the human vestibular system *Advance Otorhinolaryng 17*, 169
Tibbling, L. 1970 Observations on central mechanisms common to vestibular and optokinetic nystagmus *Acta Otolaryng* (Stockh) *69*, 434
Torok, N 1970 The effects of arousal upon vestibular nystagmus *Advance Otorhinolaryng 17*, 76
Trincker, D, Sieber, J & Partual, J 1961 Schwingungsanalyse der vestibulär, optokinetisch und durch elektrische Reizung ausgelösten Augenbewegungen beim Menschen *Kybernetik 1* 21
Wendt G R 1965 The nature of adaptation to oscillatory rotation In *The role of the vestibular organs in the exploration of space* NASA SP 77 Washington, D C 133
Young, L R., Dichgans, J, Murphy, R & Brandt, Th 1973 Interaction of optokinetic and vestibular stimuli in motion perception *Acta Otolaryng* (Stockh) *76*, 24

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# INTERACTION BETWEEN THE UTRICLES AND THE VERTICAL SEMICIRCULAR CANALS

## VI *Unilateral Selective Sectioning of the Horizontal and Vertical Ampullar Nerves, followed by Tilting around the Longitudinal Axis*

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(Received July 15 1973)

*Abstract* Unilateral selective left sided sectioning of the horizontal and vertical ampullar nerves was performed After the operation, horizontal rotatory nystagmus developed When the cats were tilted around their longitudinal axis towards the operated ear the horizontal nystagmus increased in intensity at the same time as the rotatory component disappeared Tilting towards the non-operated ear increased the rotatory nystagmus and completely inhibited the horizontal component The cause of this reaction is discussed

Earlier investigations (Fluur & Siegborn, 1973*a* *b*) have shown an intimate cooperation between the utricles and the horizontal semicircular canals It has been demonstrated that one utricle, after unilateral sectioning of the horizontal ampullar nerve and the utricular nerve, during tilting of the animal around its longitudinal axis towards the operated ear, facilitates *horizontal nystagmus released from* the ipsilateral horizontal semicircular canal Simultaneously, however, there also takes place an inhibition of the reflex arc from the contralateral horizontal semicircular canal (Fluur & Siegborn, 1973 *c*) Because electrical stimulation of a small area on the mid lateral surface of the utricle can produce horizontal nystagmus towards the stimulated ear (Fluur & Mellstrom, 1970) it can be considered as definitely established that it is this area which during tilting in the above-mentioned direction causes nystagmus Other investigations have shown that selective unilateral sectioning of the two vertical ampullar nerves gives rise to a counterclockwise rotatory nystagmus, with the upper pole of the eyes beating towards the intact ear Tilting around the animals' longitudinal axis gives rise to increased nystagmus if the animals are tilted towards the intact ear, and inhibits it if they are tilted in the opposite direction (Fluur & Siegborn, 1974)

Regarding the above mentioned experiments, it may be objected that it is not possible by means of tilting to stimulate only parts of the utricular surface, since the whole surface is influenced simultaneously by the sliding of the otolith membrane The explanations adduced for the cause of the above-mentioned reflex activity may, for the uninitiated, seem somewhat theoretical, but all the relevant facts have been stated in an earlier paper (Fluur & Mellstrom, 1970) Therefore, to make it even more clear, we intend to integrate the two experimental series with the horizontal and the vertical semicircular canals respectively and to investigate what happens when these two experiments are performed on one and the same animal

## MATERIAL AND METHOD

Eight cats were used for the experiments They were first anesthetized with ether in a so-called

on human vestibular responses *Acta Otolaryng* (Stockh ) Suppl *190*, 1
Collins W E. 1968 Special effects of brief periods of visual fixation on nystagmus and sensations of turning *Aerospace Med 39*, 257
— 1969 Influence of head position on the habituation of vertical vestibular nystagmus in the cat *J Comp Physiol Psychol 67*, 156
— 1973 Habituation of vestibular responses an overview In *Handbook of sensory physiology*, vol VI Vestibular system (ed H H Kornhuber) Springer, Berlin (in preparation)
Collins, W E & Guedry, F E, Jr 1962 Arousal effects and nystagmus during prolonged constant angular acceleration *Acta Otolaryng* (Stockh ) *54*, 349
Crampton, G H & Brown, J H 1964 Repeated vertical semicircular canal stimulation does not habituate horizontal nystagmus in cat *Acta Otolaryng* (Stockh ) *58*, 441
Dichgans, J, Schmidt, C L & Graf, W 1973 Visual input improves the speedometer function of the vestibular nuclei in the goldfish Exp Brain Res *18*, 319
Dichgans, J & Brandt, Th 1972 Visual vestibular integration and motion perception In *Cerebral control of eye movements* (ed J Dichgans & E Bizzi), p 327 (Bibl Ophthal *82*) Karger, Basel
— 1973 Optokinetic motion sickness and pseudocoriolis effects induced by moving visual stimuli *Acta Otolaryng* (Stockh ) *76*, 339
Dichgans, J, Held, R, Young, L R & Brandt, Th 1972. Moving visual scenes influence the apparent direction of gravity *Science 178*, 1217
Fischer, M H & Kornmüller, A E 1930 Optokinetisch ausgelöste Bewegungswahrnehmungen und optokinetischer Nystagmus *J Psychol Neurol* (Lpz ) *41*, 273
Fukuda, T & Tokita T 1964 Diagonal nystagmus Influence of optokinetic stimulation on postrotatory and caloric nystagmus *Confin Neurol 24* 127
Geelmakers R 1970 Vestibular adaptation in Rana *Advance Otorhinolaryng 17* 107
Groen J T 1965 Central regulation of the vestibular system *Acta Otolaryng* (Stockh ) *59* 211
Guedry, F E 1965 Psychological studies of vestibular function In *Contributions to sensory physiology* (ed W D Neff) Academic Press New York
Henn V, Young L R & Fineley, C 1973 Visual input in the nucleus vestibularis of the alert monkey *Pflügers Arch Ges Physiol*, Suppl *343*, 171
Hinoki, M & Terayama K 1966 Studies on the optokinetic nystagmus from the standpoint of body equilibrium *Acta Otolaryng* (Stockh ) *62*, 8
Hood, J D & Pfaltz, C R 1954 Observations on the effects of repeated stimulation upon rotational and caloric nystagmus *J Physiol 124* 130
Johnson, D D & Torok N 1970 Habituation of nystagmus and sensation of motion after rotation *Acta Otolaryng* (Stockh ) *69*, 206
Jung, R 1948 Die Registrierung des postrotatorischen und optokinetischen Nystagmus und die optisch vestibuläre Integration beim Menschen *Acta Otolaryng* (Stockh ) *36*, 199
Klinke, R & Schmidt, C. L. 1970 Efferent influence on the vestibular organ during active movement of the body *Pflügers Arch Ges Physiol 318*, 352
Mach, E 1875 *Grundlinien der Lehre von den Bewegungsempfindungen* Wilhelm Engelmann Leipzig (also E S Bonset, Amsterdam, 1967)
Maxwell, S S, Burke, U L. & Reston C 1922. The effect of repeated rotation on the duration of after nystagmus in the rabbit *Amer J Physiol 58*, 432
Miyoshi, T, Pfaltz, C R & Piffko P 1973 Effect of repetitive optokinetic stimulation upon optokinetic and vestibular responses *Acta Otolaryng* (Stockh ) *75*, 259
Nilsson, A 1972 Comparisons between oculogyral illusion, turning sensation and nystagmus within a process frame of reference In *Visual aftereffects and the individual as an adaptive system* (A Andersson A Willson, E Ruuth & G Smith) CWK Gleerup, Lund Sweden 1972
Pfaltz, C R & Piffko, P 1970 Studies on habituation of the human vestibular system *Advance Otorhinolaryng 17*, 169
Tibbling, L. 1970 Observations on central mechanisms common to vestibular and optokinetic nystagmus *Acta Otolaryng* (Stockh ) *69*, 434
Torok, N 1970 The effects of arousal upon vestibular nystagmus *Advance Otorhinolaryng 17*, 76
Trincker, D, Sieber, J & Partual, J 1961 Schwingungsanalyse der vestibulär, optokinetisch und durch elektrische Reizung ausgelösten Augenbewegungen beim Menschen *Kybernetik 1*, 21
Wendt G R 1965 The nature of adaptation to oscillatory rotation In *The role of the vestibular organs in the exploration of space* NASA SP-77, Washington D C 133
Young L R, Dichgans, J, Murphy, R & Brandt Th 1973 Interaction of optokinetic and vestibular stimuli in motion perception *Acta Otolaryng* (Stockh ) *76*, 24

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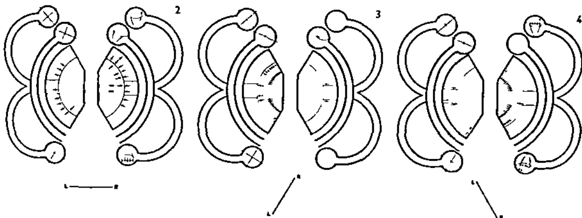



*Fig 2* Diagram of the utricles and semicircular canals in resting position after unilateral selective sectioning of the left horizontal and vertical ampullar nerve The arrows indicate the orientation of the hair cells, i.e., the direction in which they increased their discharge frequency

*Fig 3* Diagram of the utricles and semicircular canals after unilateral selective sectioning of the left horizontal and vertical ampullar nerves and when the animal is tilted toward the left. Arrow indication same as in Fig 2 The number of arrows symbolizes discharge frequency

*Fig 4* Diagram of the utricles and semicircular canals after unilateral selective sectioning of the left horizontal and vertical ampullar nerves, and when the animal is tilted to right Arrow indication same as in Fig 2 The number of arrows symbolizes discharge frequency

area of the right labyrinth and the medial area of the left labyrinth facilitating clockwise nystagmus is increased This adequately explains the total inhibition of all counterclockwise rotatory nystagmus

Tilting in the opposite direction has quite the contrary effect (Fig 4) Here, we have an inhibition of the activity of the mid lateral "horizontal" area on the utricular surface of the right ear, causing the inhibition of the horizontal nystagmus The function of the areas which give rise to clockwise rotatory eye movements is on the other hand facilitated and so is the counterclockwise rotatory nystagmus

The results obtained in the present investigation are in full agreement with clinical findings on patients with unilateral peripheral vestibular disorders described by Nylen as early as 1924

## ZUSAMMENFASSUNG

Selektive unilaterale Abschneidung der linken N ampullaris canalis horizontalis und N ampullaris canalis verticalis wurde vorgenommen Dies ergab einen horizontal rotatorischen Nystagmus Als die Katzen um ihre longitudinale Achse nach der operierten Seite hin gekippt wurden wurde die Geschwindigkeit des horizontalen Nystagmus erhoht gleichzeitig verschwand die rotatorische Komponente Eine Kippung in Richtung des nicht operierten Ohres erhohte die Geschwindigkeit des rotatorischen Nystagmus und die horizontale Komponente verschwand Die Grunde dafür wurden diskutiert

## REFERENCES

Fluur E & Mellstrom A 1970 Utricular stimulation and oculomotor reactions *Laryngoscope 80* 1970

Fluur E & Siegborn J 1973 *a* Interaction between the utricles and the horizontal semicircular canals. I Unilateral selective sectioning of the horizontal ampullar nerve followed by tilting around the longitudinal axis *Acta Otolaryng* (Stockh) *75* 17

— 1973 *b* Interaction between the utricles and the horizontal semicircular canals II Unilateral selective sectioning of the horizontal ampullar and the utricular nerve followed by tilting around the longitudinal axis *Acta Otolaryng* (Stockh) *75* 393

— 1973 *c* Interaction between the utricles and the horizontal semicircular canals. III Sectioning of the horizontal ampullar nerve on one side and of the utricular nerve on the other followed by tilting around the longitudinal axis *Acta Otolaryng* (Stockh) *75* 485

— 1974 Interaction between the utricles and the vertical semicircular canals V Bilateral selective sectioning of anterior or posterior ampullar nerves

or unilateral selective sectioning of the two vertical ampullar nerves followed by tilting around their bi temporal or longitudinal axis *Acta Otolaryng* (Stockh ) In press
Lowenstein O & Wersäll J 1959 A functional interpretation of the electron microscopic structure of the sensory hairs in the cristae of the Elasmobranch *Raja Clavata* in terms of directional sensitivity *Nature 184* 1807
Nylen C O 1924 Some cases of ocular nystagmi due to certain positions of the head *Acta Oto laryng* (Stockh ) 6, 106

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# ULTRASTRUCTURE OF MIDDLE EAR MUSCLE IN THE RABBIT

## II *Tensor Tympani*

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*Abstract* Two types of muscle fibers were observed The first was of larger diameter with relatively few mitochondria and well delineated myofibrils separated by many sarcoplasmic reticulum The myoneural junctions of these cells show well developed postsynaptic foldings The second type was of small diameter with many mitochondria and poorly separated myofibrils with sparse sarcoplasmic reticulum Their myoneural junctions show much less developed postsynaptic foldings

Two types of muscle fibers have been observed in mammalian middle ear (Erulkar et al., 1964, Fernand & Hess, 1969, Hirayama & Daly, in preparation, Seiden, 1971) and extraocular (Peachey, 1971) muscles One type is morphologically slow (Hess, 1970), the other is of the twitch type

In rabbit stapedius muscle two varieties of twitch fibers were reported, in addition to the slow fibers (Hirayama & Daly, in preparation) Functional differences have been noted between the two middle ear muscles of rabbit The tensor tympani has a higher reflex threshold smaller reflex tension and longer contraction time than the stapedius (Wersall 1959) It is thus of interest to compare these two muscles morphologically In the present study, the tensor tympani of rabbit was examined by light and electronmicroscopy

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## METHODS

The tensor tympani muscles of normal adult albino rabbits (2 5–3 Kg) were studied in frozen sections and by electronmicroscopy Serial frozen sections (20 μ) were incubated for succinic dehydrogenase activity (Nachlas et al, 1957) for study of the mitochondrial distributions (Gauthier & Padykula 1966, Gauthier, 1969) within the respective fibers Other animals were perfused with 2% glutaraldehyde and post fixed in 1% osmium tetroxide These specimens were embedded in Epon 812 and serially sectioned by steel knife at 20 μm for phase contrast viewing (Davidowitz et al, 1972) Such sections provided an initial light microscopic survey of the muscle whereby tentative fiber populations could be discriminated according to fiber diameter and mitochondrial content Selected 20 μm sections were cemented onto support blocks for further transverse sectioning at 1 μm by glass knife to provide a more detailed view of the cells and their myoneural junctions Correlated longitudinal and transverse ultrathin sections of the same cell were obtained from serial 20 μm sections by a previously described

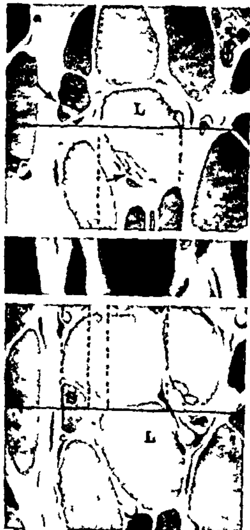






*Fig 1* Transverse Epon sect on ( m) toluid ne blue sta n Typ cal large (*L*) and small (*S*) cells are selected for further ultrath n section ng Myoneural junct ons (*arro s*) are ev dent on both of these cells The hor zontal sol d l ne *ind cates the l ne of match with* cell per meters along the upper edge of the long tud nal sect on in Fig 2 Vertical dashed l nes in d cate *the intersection of the long tud nal and trans* verse cell perimeters of the large cell (*L*) (No inter sect on w th the small cell (*S*) is ind cated insofar as *the long tudinal plane of sect on had fallen out of* that cell just before reach ng th s transverse plane) × 1 200

*Fig 2 Long tu nal 1 μm sect on cut through a* 20 μm Epon sect on Th s is a small portion of a long r bbon of many cells wh ch conta ns the pre *selected fibers L and S The l nes of per meter* match through the two transverse sect ons is deter mined by superimpos ng each edge of the ent re rib bon over *that transverse sect on which had or g nally* been cont guous with it Th s 1 μm sect on was taken immediately after the ultrath n sections shown in Figs 7 and *10 × 1 200*

*Fig 3* The second of the bracket ng trans erse sec t ons. Here the sol d l ne of match passes through the m ddle of the small cell (*S*) The upward dis- placement of the l ne of match through cells *S*" *and "L (cf Fig 1) ind cates a somewhat* obl que plane of section through these f bers For some of the cells appearing in Figs 1 and 3 there s much *less of a sh ft n relat on to the respect e* lines of match Such disparity of sh ft ng reflects a cons der able amount of f ber d splacement in relat on to *each other at the m ddle portion of th s muscle* × 1 200

*Fig 4* Same sect on as in F g 1 at higher magn f ca t on *Large fiber (L) shows sparse mitochondr al* granu larity the small fiber (*S*) conta ns a relat vely more dense mitochondr al distribut on Myoneural junct ons *are ind cated by arrows × 3 000*

*Fig 5* Succ n c dehydrogenase sta n of 20 μm frozen section The large f ber (*L*) shows sparse sta ned gran *ules the small f bers (S) conta n numerous densely* packed stained granules Presumably the granules in d cate mitochondrial locat ons × 1 200

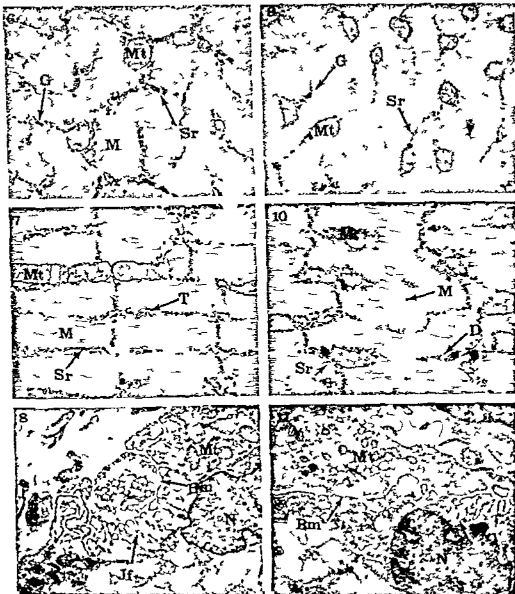



*Fig 6* Transverse section of the large fiber (*L*) indicated in Fig 1 The myofibrils (*M*) are well delineated by sarcoplasmic reticulum (*Sr*) and some glycogen granules (*G*) Few mitochondria (*Mt*) are evident × 15 000

*Fig 7* Correlated longitudinal section of the same large fiber shown in Fig 6 The myofibrils (*M*) are clearly distinguishable Mitochondria (*Mt*) are few and the transverse tubular system (*T*) is frequently seen *M* M line *Sr* Sarcoplasmic reticulum × 15 000

*Fig 8* Nerve ending from same transverse section as Fig 6 showing well developed junctional foldings (*Jf*) *Bm* Basement membrane *N* Nucleus *Mt* Mitochondria × 10 000

*Fig 9* Transverse section of the small fiber indicated in Fig 1 The myofibrils are not clearly distinguishable and blend one with the other Mitochondria (*Mt*) are numerous and little sarcoplasmic reticulum (*Sr*) is evident. *G* Glycogen granules × 15 000

*Fig 10* Correlated longitudinal section of the same small fiber shown in Fig 9 Myofibrils are poorly distinguishable and not well separated Mitochondria (*Mt*) are abundant Few diads (*D*) or triads *are seen*. M line (*M*) is occasionally discernible but indistinct. *Sr* Sarcoplasmic reticulum × 15 000

*Fig 11* Nerve ending from same transverse section as Fig. 9 showing few poorly developed junctional foldings (*Jf*) *BM* Basement membrane *N* Nucleus *Mt* Mitochondria × 10 000

procedure (Davidowitz et al, 1972) whereby the second of three consecutive 20 μm sections is sandwiched between Epon sheets to provide a ribbon of longitudinally cut fibers (Fig 2) The first and third of the three 20 μm sections provide bracketing transverse sections through the same fiber population (Figs 1 and 3) The corresponding cell identities are established along each edge of the ribbon (Fig 2) where the longitudinal cell perimeters intersect their circular counterparts in the bracketing transverse sections along a line of match (Figs 1 and 3) By monitoring through the sandwich during the longitudinal sectioning, one may observe the transverse profile of the cells, and thus aim for preselected fibers of interest Ultrathin sections were collected on Formvar grids (1 mm opening), stained with uranyl acetate and lead citrate, and examined by Siemens Elmiskop I

## RESULTS

Light microscopy of the 1 μm Epon sections revealed two clearly distinguishable fiber populations (Figs 1 and 4) The first was of larger diameter (30–40 μm) and contained relatively sparse mitochondria, the second was of smaller diameter (10–20 μm) and showed relatively numerous mitochondria These two types were evidenced as well in the frozen sections treated for succinic dehydrogenase (Fig 5) Fibers of intermediate diameter and granularity were also observed The proximal portion of the muscle is typically composed of a core of connective tissue with small cells grouped along the periphery, the distal portion of the muscle contained a mixture of the various fiber diameters

The fine structure of a typical large diameter fiber (Fig 1 L) is shown in correlated transverse and longitudinal section in Figs 6 and 7 respectively It contains distinct myofibrils separated by abundant sarcoplasmic reticulum There are few mitochondria, triads are frequently observed and the M line is clearly evident The motor nerve ending of this cell (Fig 8) shows well developed junctional foldings, and the terminal axon lies deep within an invagination of the muscle fiber

The typical small diameter fiber indicated (S) in Fig 1 is shown in correlated transverse and longitudinal ultrathin sections in Figs 9 and 10 respectively Its myofibrils are indistinct, poorly organized, and appear to fuse one with the other The sarcoplasmic reticulum is sparse, there is a moderate amount of glycogen granules, and few diads or triads are seen The mitochondria are numerous and do not tend to form clusters or chains An indistinct M line is occasionally discernible The myoneural junction of this fiber (Fig 11) shows a relatively poor development of the junctional folding, and the terminal axon lies superficially on the surface of the muscle fiber No muscle spindles were observed

## DISCUSSION

The small diameter fibers observed in this study display poorly delineated myofibrils and sparse sarcoplasmic reticulum Such fibers have been referred to as Felderstruktur, and are thought to be physiologically slow (Hess 1970) The appearance of this fiber is consistent with that of slow fibers observed in the tensor tympani of guinea pig (Seiden 1971), and cat (Erulkar et al, 1964, Fernand & Hess, 1969), and in the stapedius of rabbit (Hirayama & Daly, 1973), and cat (Fernand & Hess, 1969) The moderate junctional folding of this fiber, however, appears to be somewhat more developed than previously indicated for slow fibers in the middle ear muscles (Fernand & Hess, 1969, Hirayama & Daly in preparation, Seiden, 1971)

The large diameter fiber observed in this study appears similar to the first type of twitch fiber (white) reported in stapedius muscle of rabbit (Hirayama & Daly, 1973), though we did not observe fibers which could be clearly classified as being of the second (red) twitch type reported in that study

## ACKNOWLEDGMENT

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## ZUSAMMENFASSUNG

Zwei Arten von Muskelfasern wurden beobachtet Die erste Art war von grosserem Durchmesser mit verhaltnismassig wenigen Mitochondrien und deutlich ausgepragten Myofibrillen, getrennt durch zahlreiches sarkoplasmatisches Retikulum Die myoneuralen Verbindungen dieser Zellen haben gut entwickelte postsynaptische Falzen Die zweite Art war von kleinerem Durchmesser mit vielen Mitochondrien und kaum getrennt durch Myofibrillen mit sparlichem sarkoplasmatischem Retikulum Ihre myoneuralen Verbindungen wiesen viel weniger entwickelte postsynaptische Falzen auf

## REFERENCES

Davidowitz J, Pachter B Brady, G & Greinin, G M 1972 Steel knife sectioning of epon blocks as applied to the study of muscle In *30th Ann Proc Electron Microscopy Soc Amer* (ed C. J Apceneaux) Los Angeles, Calif

Erulkar, S D Shelanski, M L, Whitsel, B L & Ogle D 1964 Studies of muscle fibers of the tensor tympani of the cat *Anat Rec 149* 279

Fernand, V S V & Hess, A 1969 The occurrence structure and innervation of slow and twitch muscle fibers in the tensor tympani and stapedius of the cat. *J Physiol 200*, 547

Gauthier, G F 1969 On the relationship of ultrastructural and cytochemical features to color in mammalian skeletal muscle *Z Zellforsch 95* 462

Gauthier, G F & Padykula, H A 1966 Cytological studies of fiber types in skeletal muscle *J Cell Biol 28*, 333

Hess, A 1970 Vertebrate slow muscle fibers *Physiol Rev 50*, 40

Hirayama, M & Daly, J F 1973 Ultrastructure of middle ear muscle in the rabbit I Stapedius Muscle *Acta Otolaryng* (Stockh) *77* 13

Nachlas, M M, Tsou, K DeSouza, E, Cheng C & Seligman, A 1957 Cytochemical demonstration of succinic dehydrogenase by the use of a new *p*-nitrophenyl substituted ditetrazole *J Histochem Cytochem 5*, 420

Peachey, L 1971 The structure of the extraocular muscle fibers of mammals In *The control of eye movements* (ed P Bach y Rita C C Collins and J E Hyde) Academic Press, New York.

Seiden, D 1971 Slow muscle fibers in the tensor tympani muscle of the guinea pig *Amer J Anat 132* 267

Wersall, R 1959 The tympanic muscles and their reflexes *Acta Otolaryng* (Stockh), Suppl *139*

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# ULTRASTRUCTURE OF THE BASILAR PAPILLA, AN AUDITORY ORGAN IN THE BULLFROG

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*Abstract* The basilar papilla is one of two auditory organs found in the frog s inner ear Examination of the papilla in the bullfrog, *Rana catesbeiana*, by light and electron microscopy has confirmed earlier descriptions of its gross morphology and revealed new details of its fine structure The papilla is a tubular evagination of the saccule, terminating in a thin contact membrane which separates endolymphatic and perilymphatic spaces Its sensory epithelium occupies a crest extending halfway around the tube and is surmounted by a tectorial membrane Between 300 and 500 myelinated nerve fibers synapse with about 60 hair cells Extending from the hair cells are sensory hair bundles, lodged in canals in the tectorial membrane Each bundle consists of a group of stereocilia and a single asymmetrically placed kinocilium located on the side of the bundle facing the contact membrane Each hair cell synapses at its base with several afferent terminals but efferent synapses ave not been found

The basilar papilla is one of two auditory receptor organs found within the labyrinth of anuran amphibian species (Fig 1) Both basilar and amphibian papillae have been described in light microscopic detail by a number of investigators (van Bergeijk & Witschi, 1957, Geisler et al, 1964) and their response characteristics have been studied by electrophysiological techniques in several species (Frishkopf & Goldstein, 1963, Sachs, 1964 Capranica & Frishkopf, 1966, Frishkopf & Geisler 1966) The purpose of the present report is to describe the ultrastructure of the basilar papilla in functional terms A preliminary report of this work has been published elsewhere (Frishkopf & Flock, 1967)

This research was done while both authors were at Bell Laboratories Murray Hill New Jersey One of us (Å F) received support from the Swedish Medical Research Council (Grant no 14x 2461)

## MATERIALS AND METHODS

Young adult bullfrogs, *Rana catesbeiana*, body weight 30–100 g, were anesthetized by intra muscular injection of DIAL in urethane (CIBA), dosage 2 cc/kg, an incision was made in the skin along the dorsal midline, starting anterior and ending posterior to the external eardrum A skin flap formed by cutting transversely at each end of the midline incision was removed close to the superior edge of the tympanic ring The dorsal fascia was cut close to its line of medial attachment and the muscles beneath detached and retracted to expose the surface of the otic capsule Laterally, the outlines of the anterior and posterior semicircular canals form a right angle, with the vertex directed medially

The bone over and between the canals was thinned with a dental drill and removed by cutting with a razor or fine scissors, exposing the perilymphatic membranes Blood flow may be observed in several small vessels in the walls of the labyrinth An opening was made into the saccule, identified by the large white otolith it contains, through perilymphatic and endolympatic membranes A second opening into the endolymphatic system was made at the top of the common crus, where the vertical canals meet

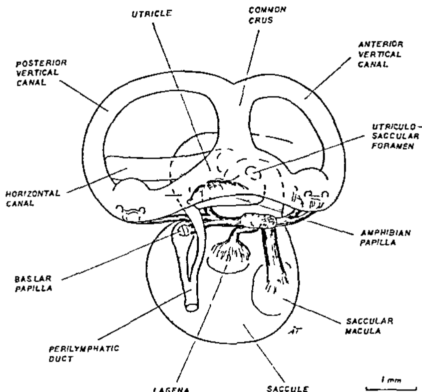



*Fig 1* Schematic drawing of left labyrinth of bullfrog, from medial, showing endolymphatic system, sensory organs and their innervation Perilymphatic duct is shown in relation to auditory papillae Remainder of perilymphatic system, utricular macula and its innervation are omitted

With a narrow tipped glass pipette, cold fixative was flushed through the saccule, emerging through the crus The fixative was either 1% $OsO_4$ in veronal acetate buffer or 3-6% glutaraldehyde in phosphate buffer Often one labyrinth was fixed in $OsO_4$ and the other in glutaraldehyde The otic capsules were excised, transferred to fixative and the labyrinths rapidly removed and opened further The saccular otolith was removed and fixation allowed to proceed for 1 hour The tissue was washed in cold buffer and, with glutaraldehyde fixation, postfixed in phosphate buffered $OsO_4$, dehydration in increasing alcohols and propylene oxide and embedding in epon followed Further dissection of the individual organs was carried out in buffer or in alcohol Thick sections (3$\mu$) were viewed unstained or stained with toluidine blue in phase contrast Thin sections were cut on an LKB Ultrotome II, at 600–1 000 Å, stained with uranyl acetate and lead citrate, and examined in a Hitachi HS-7S electron microscope

## RESULTS

The basilar papilla is a tubular evagination of the posterior wall of the saccule (Figs 1 and 2*a*) The tube terminates in a thin contact membrane which separates the endolymphatic and perilymphatic systems The sensory epithelium consists of several parallel rows of hair cells, each row arranged semicircumferentially around the perimeter of the tube (Fig 2*b*) The sensory crest (Figs 2*a*, 3, 4) rises abruptly 60 $\mu$m from the contact membrane, reaches a height of about 30 $\mu$m, and gradually diminishes over a distance of 100 $\mu$m Approximately 5 or 6 rows of sensory cells are seen, separated by supporting cells whose darkly staining nuclei lie close to the basement membrane An earlier study (Geisler et al, 1964) has placed the number of hair cells in the papilla at about 60, in substantial agreement with our estimates Hair bundles project from some of the hair cells, over them is the tectorial membrane, here shrunken and partially detached

*Fig. 2.* (*a*) Longitudinal section of basilar papilla. Contact membrane separates perilymphatic space on left from endolymphatic space on right. Tectorial membrane, overlying sensory crest, extends halfway across the lumen and bends near its top toward saccule. Myelinated nerve fibers approach papilla from below. Small blood vessels are seen in the cartilagenous wall. (*b*) Cross section of papilla through sensory epithelium. Sensory crest consists of hair cells and supporting cells, some of whose nuclei are seen; several hair bundles are evident. Tectorial membrane extends across lower half of tube and is attached to walls of papilla.

In (*a*) and (*b*) open spaces occur in tectorial membrane; its detachment from sensory epithelium is probably due to shrinkage following fixation and dehydration. Nerve branch to papilla divides into smaller bundles and fibers lose their myelination just before entering sensory epithelium.




*Fig. 3.* Longitudinal section of papilla at higher magnification (cf. Fig. 2*a*). Sensory crest, tectorial membrane, and part of contact membrane are seen. Nerve fibers approach sensory epithelium from below. Hair cell nuclei and hair bundles are evident near top of sensory crest. Nuclei below belong to *supporting cells*.

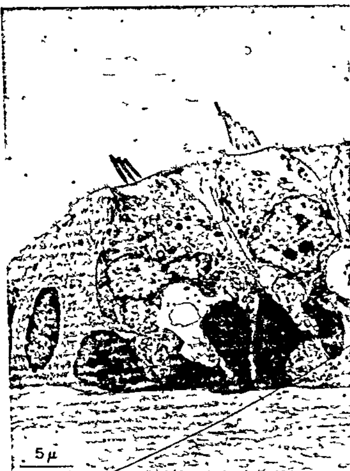



*Fig 4* Two hair cells, corresponding to a portion of Figs 2*a*, 3 Stereocilia arise from cuticular regions of cells Hair cells are separated by supporting cells from whose apical ends microvilli extend Hair bundles were probably enclosed within spaces in tectorial membrane, now detached over most of its width Cytoplasm of hair cells is distinguished by a variety of inclusions, not present in supporting cells Nerve terminals containing mitochondria are seen between supporting cells abutting the basal ends of hair cells Open spaces may be fixation artifacts

by fixation The hair bundles extend up into recesses in the tectorial membrane as suggested in Fig 3 by the bundle at left The tectorial membrane appears to be firmly attached to the supporting cells just to the left of the first row of hair cells At its upper free end the tectorial membrane is bent toward the saccule (Fig 2*a*) and is firmly attached at its margins to the wall of the papilla over most or all of this region of bending (Fig 2*b*)

The nerve bundle to the basilar papilla forms part of the posterior branch of the eighth nerve (Geisler et al, 1964) From the papilla the bundle courses toward the midline and then turns rostrally and after a short distance (200 μm) joins the passing bundle from the posterior vertical canal Branchlets from the amphibian papilla and lagena subsequently join the bundle to form the posterior branch of the eighth nerve, which exits from the otic capsule through the posterior foramen Within and close to the foramen is the posterior ganglion of the eighth nerve, containing the bipolar cell bodies of all the nerve branchlets including those of the basilar papilla The nerve fibers thereafter take a medial course and enter the medulla, where they terminate

In Fig 2*b* the myelinated nerve bundle approaches the sensory epithelium and divides into three or four smaller branches The myelin sheath ends and the unmyelinated fibers pass through the basement membrane, to terminate on the hair cells (Figs 2–4) The fibers range in size from less than 1 μm to about 6 μm (Geisler et al, 1964) and number between 300 and 500 The nerve bundle flattens as it approac

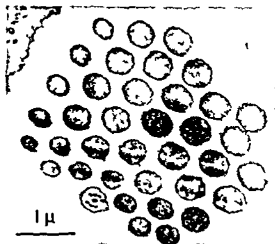



*Fig 5* Cross section of hair bundle just above hair cell Kinocil um is located to one side of bundle of stereocil a

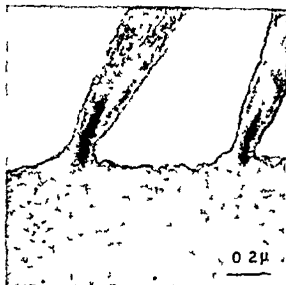



*Fig 6* Long tud nal section of stereoc l a entering cuticle. Cilia narrow as they approach cell and m crof laments converge to form a dense bundle which penetrates the cuticle for a short d stance

sensory epithelium as can be seen by comparing Figs *2a* and *2b* The plane of the ring of the sensory epithelium (Fig *2b*) lies very close to a cross section through the animal The tube of the basilar papilla is directed posterior and slightly lateral

Hair cells are found in auditory vestibular and lateral line organs A hair bundle consisting of a single kinocilium located to one side of a number of stereocilia protrudes from the apical end of each cell (Fig 5) The kinocilium is identified by a pattern—common to motile and sensory cilia—of nine double filaments surrounding a central pair of filaments The stereocilia are arranged in a series of shallow ι s behind the kinocilium The axis of symmetry of this pattern passes through the kinocilium The stereocilia decrease in height along this axis away from the kinocilium (Fig 4) Stereocilia in rows parallel to this axis show a similar decrease in height Missing elements in the pattern may result from the fact that the plane of the section failed to intercept several of the smaller stereocilia The ι closest to the kinocilium contains five stereocilia instead of seven In Fig 6 two stereocilia are partially seen in longitudinal section They contain longitudinal filaments (Fig 5) which converge near the cell surface and extend into the cuticle of the hair cell a non cytoplasmic plate found at the apical end of the cell (Fig 4) The cuticle is notched and the kinocilium arises from the cytoplasm within the notch (Fig 7)

Our data show that the kinocilium is regularly located on the side of the hair bundle closest to




*Fig 7* Long tud nal sect on through ap cal port on of ha r cell showing kinoc l um and several stereoc l a. W th n kinocil um several m crotubules are evident, extend ng into hair cell K nocil um enters cytoplasm c portion of cell conta ning a var ety of ves cles and other inclus ons To the left is the cut cle



*Fig 8* Afferent synapse of nerve terminal (right center) on hair cell (left) Dendrite contains many mitochondria Within hair cell are a variety of membrane bounded and electron dense structures mitochondria, vesicles myelin figures and dense inclusions At upper left is a portion of hair cell nucleus Synaptic region of hair cell is characterized by dense synaptic body surrounded by clear vesicles of uniform size Both pre- and postsynaptic membranes are thickened

the contact membrane and most distant from the saccular opening of the papilla This is suggested by the fact that in Figs 3 and 4 the hair bundles are longest on the side facing the contact membrane

Afferent synapses are found on the hair cells (Fig 8) The synaptic region of the hair cell contains a dense, spherical synaptic body, approximately 0 25 μm in diameter, surrounded by a large number of synaptic vesicles (diameter about 400 Å) The presynaptic membrane is thickened, troughs appear in the membrane under the synaptic body and contain dense rod like structures (Fig 9) The synaptic body is flattened on the side facing the membrane Post synaptically the membrane is also thickened and the afferent nerve terminals contain numerous vesicles and mitochondria

Each hair cell synapses with several terminals (Fig 10) and one dendrite may branch and innervate several hair cells (Fig 11) We have estimated that there are approximately 60 hair cells innervated by 300 to 500 nerve fibers, so that each hair cell must synapse on average with more than five neurons This result may be compared to the recent finding of Spoendlin (1971) that in the mammalian cochlea each inner hair cell is exclusively innervated by about 20 nerve fibers

Efferent synapses described for other organs

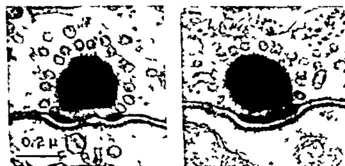



*Fig 9* Sections through afferent hair cell–dendrite synapse Hair cell contains synaptic body and vesicles above dense rod like structures which lie in troughs in presynaptic membrane Sections are from different synapses but are probably orthogonal cuts through similar structures

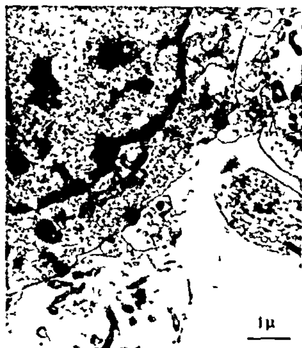


*Fig 10* Hair cell with several afferent synapses each identified by synaptic body vesicles and thickened membranes

of the acustico lateralis system (Wersäll et al, 1965) have not been observed in the basilar papilla (Frishkopf & Flock 1967) Such synapses are characterized by a large number of densely packed vesicles within the nerve terminal close to the presynaptic membrane and by a closely apposed membranous sac within the hair cell Although we searched for such structures we found only the type of synapse described above, corresponding closely to afferent hair cell-dendrite synapses found in other auditory and vestibular organs (Wersäll et al, 1965)

## DISCUSSION

The input to the auditory papillae takes place by way of the tympanum, middle ear bones, and perilymphatic system The latter is a membrane limited fluid filled space which envelops the endolymphatic system, in the region of the basilar and amphibian papillae the two systems are separated only by thin contact membranes (Figs 1 and 2*a*) Movement of the perilymph displaces the contact membrane and endolymph. The resulting tectorial membrane displacement acts through the sensory hair bundles to stimulate hair cells and nerve fibers

A relation is known to exist in hair cells of the vertebrate acustico-lateralis system between the orientation of the sensory hair bundle as determined by the asymmetric location of the kinocilium and the direction of motion of the overlying structure (cupula or tectorial membrane) that is effective in stimulating the organ (for review see Flock, 1971) It has been shown for example that in the semicircular canals



*Fig 11* Afferent nerve terminal synaptically contacting two hair cells That these are in fact two hair cells was verified by examining a larger region of the section

utriculo petal motion of the cupula increases the activity of nerves from the horizontal canal and decreases the activity from the vertical canals, utriculo fugal motion has the opposite effects Examination of the orientation of the hair bundles with the electron microscope reveals that in the horizontal canal the kinocilium is located on the side of the hair bundle closest to the utricle while in the vertical canals it is located on the side of the bundle farthest from the utricle (Löwenstein & Wersäll, 1959) Thus it appears that displacement of the cupula in the direction from stereocilia to kinocilium increases—and opposite displacement decreases—neural firing rates

A relation between orientation and functional properties has also been found in the lateral line canal organs of fish (Flock & Wersäll, 1962), where the gross electrical response from the organ to a sinusoidal stimulus contains a predominant second harmonic component Electron microscopy reveals that neighboring hair bundles within the same organ are oppositely oriented It is conjectured that oppositely oriented hair cells produce electrical responses that are 180° out of phase, summed over many cells the fundamental component cancels and only higher harmonics remain A correlation between hair cell orientation, microphonic response, and nerve activity has also been found in the saccule of goldfish (Furukawa & Ishii, 1967*a*, *b*)

In the basilar papilla we have found that in each hair cell the kinocilium is located on the side of the hair bundle facing the contact membrane Therefore, displacement of the tectorial membrane along the axis of the papilla should stimulate the organ, this is the direction in which the tectorial membrane receives the maximum input from displacement of the contact membrane as well as the direction in which it presents maximum area (Fig 2) We also expect displacement of the tectorial membrane toward the contact membrane to increase—and opposite displacement to decrease—the activity in afferent nerve fibers from the papilla

On the basis of our observations, there appear to be no efferent terminals synapsing on hair cells of the basilar papilla Independent corroboration of this result has been obtained by degeneration studies (Robbins et al, 1967), from these it appears that of the labyrinth organs in the frog only the basilar papilla lacks efferent innervation Moreover, of all the organs of the acustico lateralis system so far examined, the basilar papilla is the only one that is so deficient

It is interesting to note that whereas responses of basilar papilla afferents to acoustic stimuli are exclusively excitatory, the responses of amphibian papilla afferents to one tone can be inhibited by a second tone of higher frequency (Frishkopf & Goldstein, 1963) The possible role of efferents in this inhibitory process was previously discounted, based on the persistence of the effect after sectioning the eighth nerve It is nevertheless intriguing to observe that of the two auditory organs found in amphibians only the one with efferent innervation exhibits inhibitory interactions The possibility remains that efferent terminals may be involved in a local inhibitory process This hypothesis may be tested by observing if inhibition is present at a time after cutting the nerve when efferent terminals have degenerated

It is also noteworthy that the afferent nerve fibers from both basilar and amphibian papillae exhibit threshold vs frequency functions that rise steeply at about 100 dB/octave (Frishkopf & Goldstein, 1963) The origin of such steep tuning curves is uncertain, we can now be sure, however, that in the case of the basilar papilla efferents are not involved

We have pointed out that the number of afferent nerve fibers to the basilar papilla greatly exceeds the number of hair cells therefore the terminals synapsing on each hair cell must in general derive from several fibers The innervation pattern is in that respect somewhat similar to that of inner hair cells in the organ of Corti (Spoendlin 1971) The significance and function of this highly divergent innervation pattern is not known either in the basilar papilla or in the cochlea but it could serve to increase the dynamic range of the organ

## ZUSAMMENFASSUNG

Die Papilla Basilaris ist eines zweier Gehörorgane, die im Innenohr des Frosches gefunden werden Licht und elektronenmikroskopische Untersuchungen der Papilla im Ochsenfrosch, *Rana Catesbeiana*, haben fruhere Beschreibungen ihrer grundsatzlichen Morphologie bestatigt und ausserdem neue Einzelheiten ihrer Feinstruktur aufgedeckt

Die Papilla ist ein zylindrischer Auswuchs des Sacculus und erstreckt sich bis zu einer dünnen Kontaktmembran, welche die endolymphatischen von den perilymphatischen Räumen trennt Das sensorische Epithelium uberdeckt kammformig ein Halbrund des Zylinders, und es wird uberhangen von einer Tectorial membran Ungefahr 300 bis 500 myelinierte Nervenfasern haben synaptischen Kontakt mit ungefahr 60 Haarzellen Sensorische Haarbuschel entspringen von den Haarzellen und werden von Kanalen in der Tectorialmembran aufgenommen Jedes Haarbundel besteht aus einer Gruppe von Stereocilien und einem einzigen Kinocilium, welches asymmetrisch der Haarzelle aufsitzt und zwar auf jener Seite des Haarbündels, die der Kontaktmembran zugewandt ist An der Basis hat jede Haarzelle synaptischen Kontakt mit mehreren afferenten Nervenendigungen, efferente Synapsen konnten nicht gefunden werden

## REFERENCES

Capranica, R R & Frishkopf, L S 1966 Responses of auditory units in the medulla of the cricket frog *J Acoust Soc Amer 40*, 1263

Flock, Å 1971 Sensory transduction in hair cells In *Handbook of sensory physiology*, Vol 1 Principles of Receptor Physiology (ed W R Löwenstein), pp 396–441 Springer-Verlag, Berlin

Flock, Å & Wersäll, J 1962 A study of the orientation of the sensory hairs of the receptor cells in the lateral line organ of fish, with special reference to the function of the receptors *J Cell Biol 15*, 19

Frishkopf, L S & Flock, Å 1967 Ultrastructure of the basilar papilla in the bullfrog *J Acoust Soc Amer 41*, 1578

Frishkopf, L S & Geisler, C D 1966 Peripheral origin of auditory responses recorded from the eighth nerve of the bullfrog *J Acoust Soc Amer 40*, 469

Frishkopf, L S & Goldstein, M H, Jr 1963 Responses to acoustic stimuli from single units in the eighth nerve of the bullfrog *J Acoust Soc Amer 35*, 1219

Furukawa, T & Ishii, Y 1967*a* Neurophysiological studies on hearing in goldfish *J Neurophysiol 30*, 1377

— 1967*b* Effects of static bending of sensory hairs on sound reception in the goldfish *Jap J Physiol 17*, 572

Geisler, C D, van Bergeijk, W. A & Frishkopf, L S 1964 The inner ear of the bullfrog *J Morph 114*, 43

Harris, G G, Frishkopf, L S & Flock, Å 1970 Receptor potentials from hair cells of the lateral line *Science 167*, 76

Löwenstein, O & Wersäll, J 1959 A functional interpretation of the electron microscopic structure of the sensory hairs in the cristae of the elasmobranch *Raja clavata* in terms of directional sensitivity *Nature* (Lond) *184*, 1807

Robbins, R G, Bauknight, R S & Honrubia, V 1967 Anatomical distribution of efferent fibers in the VIIIth cranial nerve of the bullfrog (*Rana catesbeiana*) *Acta otolaryng* (Stockh) *64*, 436

Sachs, M B 1964 Responses to acoustic stimuli from single units in the eighth nerve of the green frog *J Acoust Soc Amer 36*, 1956

Spoendlin, H 1971 Degeneration behaviour of the cochlear nerve *Arch Klin Exp Ohr Nas Kehlkopfheilk 200*, 275

van Bergeijk, W A & Witschi, E 1957 The basilar papilla of the anuran ear *Acta Anat* (Basel) *30*, 81–91

Wersäll, J, Flock, Å & Lundquist, P G 1965 Structural basis for directional sensitivity in cochlear and vestibular sensory receptors *Cold Spr Harb Symp Quant Biol 30*, 115

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# AN ELECTRON MICROSCOPIC STUDY OF ADRENERGIC INNERVATION IN THE COCHLEA

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*Abstract* By using electron microscopic techniques including the administration of a "false transmitter" it has been possible to identify adrenergic terminals filled with characteristic dense-cored vesicles in the cochlea A continuous sympathetic perivascular plexus is found up to the limit of the habenula region Adrenergic terminals are also found independent of blood vessels and this innervation is especially rich in the habenula region Terminals are frequently found just before the demyelinization zone and they surround the afferent nerve bundles when they have lost their myelin sheaths In the habenula terminals are seen to make direct contact with non myelinated afferent nerve fibres Clusters of terminals are found in the tissue near the inner sulcus and toward the scala tympani No adrenergic nerve terminals were found in the organ of Corti or vascular stria The morphological localization of the sympathetic terminals support an influence on sound perception either via a direct action on sensory neurons or by vasomotor control

The possible influence of the sympathetic nervous system on the cochlear and vestibular parts of the inner ear has been the subject of much interest from clinicians as well as anatomists and physiologists An imbalance of the autonomic nervous system, in particular an overaction of the sympathetic part, has been discussed as a possible cause of diseases like Meniere s disease, tinnitus and perceptive deafness Tympanosympathectomy (Lempert 1946, Rosen, 1951) and cervical sympathectomy (Passe, 1953, Lewis, 1964) have been performed in these conditions, occasionally with good results However, very little is known about the sympathetic innervation of the inner ear in man and the knowledge of the sympathetic innervation and its function in animals is limited

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The distribution of adrenergic nerve fibres in the cochlea has been difficult to investigate because of the special anatomy of the inner ear It is enclosed in the temporal bone, and bony lamellae are closely adjacent to the innervated structures Species differences, as well as considerable individual variation within species, have been claimed (Seymour & Tappin, 1951)

Using silver impregnation methods, a perivascular plexus plus the "plexus marginalis", consisting of non myelinated fibres have been found in the cochlea (Lorente de No, 1926, Livan & Del Bo, 1951, Palumbi, 1954, Andrzjewski, 1955, 1956) The origin of these fibres was discussed and they were suggested to be of cerebral character or originating from sympathetic cervical ganglia The results of these investigations were difficult to evaluate because it was almost impossible to distinguish between sympathetic nerve fibres and other thin non myelinated nerve fibres Mapping of the sympathetic innervation was made possible with the histochemical fluorescence method for catecholamines according to Falck and Hillarp (Falck et al, 1962) Using this method, Spoendlin & Lichtensteiger (1966, 1967) traced these fibres from the cervical ganglion to the inner ear via the tympanic

plexus or the auricular branch of the vagus nerve A dense plexus in the habenula region is of special interest since nerve action potentials are thought to be initiated in the habenula at the point where myelinization starts However, with light microscopy it is not possible to determine the exact location of the adrenergic terminals In the electron microscope Spoendlin (1966) described what he supposed to be adrenergic terminals in this region Electron microscopic studies by Terayama et al (1968) did not support the presence of an adrenergic innervation in the habenula region and they concluded that the sympathetic innervation of the cochlea is confined to the vascular bed Adrenergic fibres found between the myelinated nerve fibres, independent of blood vessels, were considered to run from one blood vessel to another Special staining techniques are now available which make it possible to specifically identify biogenic amines at the ultrastructural level (Richardson, 1966, Tranzer & Thoenen, 1967) It is thus now possible to reliably identify fibres containing biogenic amines, and distinguish them from other nerve terminals The loading of adrenergic fibres with a false transmitter, such as 5 hydroxydopamine (5-OH DA), enhances the reliability of this method It was the purpose of this investigation to localize the monoamine neurons in the cochlea and to describe their ultrastructural relationship to afferent nerve fibres and blood vessels, especially in the habenula region

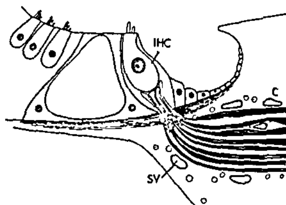



*Fig 1* Schematic diagram illustrating the adrenergic innervation of the cat cochlea *IHC*, inner hair cell *SV*, spiral vessel of the tympanic lip *C* capillary O, adrenergic nerve fibre

## MATERIAL AND METHOD

Ten healthy cats weighing from 1 0–3 7 kg were used for the experiments They were given either 5 OH DA (3,4 5-trihydroxyphenyl ethylamine) 4 × 20 mg/kg or 5 OH Dopa 3 × 200 mg/kg over a period of 48 hours (Tranzer & Thoenen 1967) In a control animal unilateral cervical sympathectomy was performed about 3 weeks before it was sacrificed The animals were anaesthetized with 2 5% Nembutal before decapitation The cochleas were rapidly dissected out and opened widely to obtain good contact with the fixation medium Fixation was performed in ice cold 3% potassium permanganate (0 1 M sodium phosphate buffer, pH 7 0) (Richardson 1966, Hokfelt & Jonsson, 1968, Hokfelt, 1969) After rinsing in Ringer solution the pieces were contrasted en bloc in 1% uranyl acetate-Ringer solution In several cases the cochlea on one side was fixed in 3% glutaraldehyde (0 133 M sodium phosphate buffer pH 7 3) followed by Veronal acetate buffered 1% osmium tetroxide solution (Rhodin 1954) and in a few cases fixation was obtained by vascular perfusion, first with Ringer solution, then with glutaraldehyde The pieces were dehydrated in ethanol and embedded in Epon (Luft, 1961) For electron microscopy, sections were cut with glass knives on an LKB Ultrotome Sections from specimens fixed in potassium permanganate were stained with lead citrate (Watson 1958) and those fixed in osmium tetroxide were stained with uranyl acetate (Reynolds, 1963) followed by lead citrate Sections were taken from the modiolus spiral ganglion, spiral osseous lamina habenula, organ of Corti, and from the stria vascularis and were examined in a Siemens El miskop I electron microscope



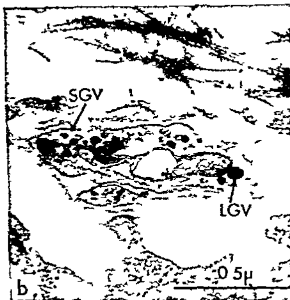




*Fig 2* Adrenergic nerve terminals have different appearance depending on fixation procedure Pretreatment with 5-OH DA (*a*) Fixation in potassium permanganate The vesicles have a distinct dense core ×51 000 (*b*) After fixation in glutaraldehyde and osmium tetroxide the vesicles are completely filled with an osmiophilic substance ×42 500 *LGV* large granular vesicle *SGV* small granular vesicle

## RESULT

In the cochlea two systems of adrenergic innervation were found one along blood vessels and one consisting of terminals freely distributed in the modiolus, spiral ganglion, spiral lamina and in the habenula The distribution pattern is illustrated in Fig 1

### *Appearance of terminals*

As in other areas the adrenergic nerve fibres of the ear are characterized by the presence of local swellings of typical appearance so-called varicosities which are found along the thin non myelinated axon in increasing numbers towards its periphery Each varicosity is named a terminal even though it does not usually terminate the axon Generally only the terminals are seen in a single section, but in serial sections the interconnecting nerve fibre is observed Each terminal contains an abundance of dense-cored vesicles the centre of which stains characteristically after fixation with potassium permanganate (Fig 2 *a*) (Richardson, 1966 Hokfelt 1969) The diameter of the vesicles is about 500 Å but a few with a diameter of 1 000 Å are also encountered Most of the vesicles appear empty if the animal is not pretreated with the false transmitter 5 OH DA (or its precursor 5 OH Dopa) The number of vesicles differs considerably between terminals, so while some are densely packed others may contain only a few This difference is independent of pretreatment with 5 OH DA or 5 OH Dopa After fixation with glutaraldehyde and osmium tetroxide the vesicles appear almost filled with a dense osmiophilic material (Fig 2 *b*) (Tranzer & Thoenen, 1967)

### *Perivascular innervation*

An adrenergic perivascular plexus was found in sections from the *modiolus spiral ganglion spiral lamina* and around the *spiral vessel of the tympanic lip* (Figs 3 and 4) Terminals close to blood vessels were usually




*Fig 3* Blood vessel in the spiral ganglion with adrenergic innervation Fixed in osmium tetroxide *CL* capillary lumen *EC* endothelial cell *NT* adrenergic nerve terminal ×46 500

not completely covered by a Schwann cell and were situated at varying distances from the vessels The distances were sometimes as small as 1 000 Å or less but very often the *terminals were situated at distances of 3 000 Å* or more Generally there were several nerve terminals situated at different distances from the vessel In potassium permanganate fixed material a second type of nerve ending containing non granulated small vesicles of smaller diameter was sometimes observed in the vicinity of adrenergic terminals close to blood vessels

In the *stria vascularis* no adrenergic terminals or cells containing catecholamines could be found either around blood vessels or elsewhere in the tissue

*Blood vessel independent innervation*

*Spiral ganglion* In the spiral ganglion adrenergic terminals were found independent of blood vessels Such terminals were seen in close proximity to myelinated ganglion cells (Fig 5) There was no special sympathetic innervation of type II ganglion cells of the non myelinated type (Spoendlin 1971) Axons and terminals

*Fig 4* Blood vessels (*Bl*) in the spiral lamina also have adrenergic innervation Fixed in potassium permanganate *CL* capillary lumen *EN* endothelial nucleus, *NT* adrenergic nerve terminal ×43 000




*Fig 5* An adrenergic nerve terminal (*NT*) between two myelinated ganglion cells (*GC*) in the spiral ganglion Potassium permanganate, ×27 000




Fig 6 a Section from the cochlea with the demyelinization zone and inner hair cell (*IHC*) Fixed in osmium tetroxide *SI* spiral vessel of the tympanic lip × 3 400

were ensheathed by Schwann cells which left part of the membrane of the terminals free

*Lamina spiralis* On their course towards the organ of Corti afferent nerve fibres lost their myelin sheath in the region of the habenula perforata where bundles of fibres left the lamina spiralis ossea Just before the demyelinization zone free terminals were seen above and below the myelinated nerve fascicles and also interspersed between the fibres Adrenergic terminals and axons usually appeared without a Schwann cell envelope, but might sometimes be totally or partly surrounded by Schwann cells

In the habenula where demyelinization occurred, bundles of axons were surrounded by adrenergic terminals Terminals were constantly found below (Figs 6 and 7) and between the nerve fibres (Fig 8) In between the unmyelinated part of the axons slender terminals were sometimes observed, containing the typical dense-cored vesicles Some terminals were located in direct contact with the non myelinated part of the axons (Fig 9) Morphological evidence of a direct synaptic contact with pre and post synaptic membrane specialization, between adrenergic terminals and afferent dendrites was not obtained Terminals in this region were generally completely free of a Schwann cell envelope Free terminals, or even clusters of terminals were also present at a greater distance from sensory fibre

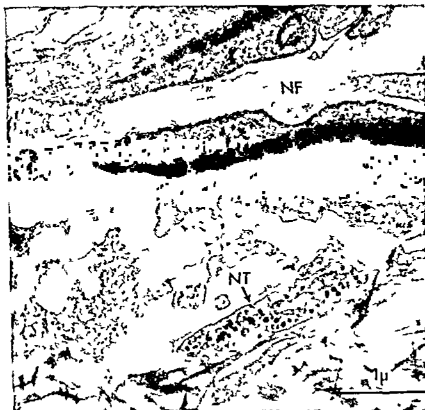



*Fig 6b* Higher magnification of the framed area in Fig 6a showing the relationship between a nerve terminal (*NT*) and demyelinating nerve fibre (*NF*) ×25 300

bundles namely above the fibres in the connective tissue below the tympanic lip of the limbus and below the fibres in the connective tissue towards the scala tympani (Fig 1) Terminals were also seen between the extension of fibroblasts below the basement membrane at the level of the inner hair cell (Fig 10)

*Organ of Corti* Adrenergic terminals were not found in the inner spiral plexus below the inner hair cells, in the tunnel of Corti, or at the level of the outer hair cells

The unilaterally sympathectomized cat got a paralysed nictitans membrane on the operated side No adrenergic terminals were found anywhere in the cochlea on that side On the non-operated side there was a normal distribution of adrenergic terminals

## DISCUSSION

Since the introduction of the fluorescence method by Falck and Hillarp (Falck et al, 1962) the cochlear sympathetic innervation has been investigated by fluorescence microscopy Having found fluorescent sympathetic nerves both close to, and apart from blood vessels, the question has been to what extent the blood vessels in the cochlea are innervated and whether there is a system of terminals independent of blood vessels Spoendlin & Lichtensteiger (1967) showed a perivascular plexus out to the periphery of the modiolus Terayama et al (1965, 1966) found fluorescence around the spiral vessel too Electron microscopic studies using standard osmium fixation have also been performed, where unmyelinated nerve fibres have been mistaken for adrenergic nerve fibres (Ross, 1971) Terayama et al (1968) described terminals around blood vessels in the radiating cochlear bundles However, they found none around the spiral vessels or in the habenula region

It has been shown (Hökfelt & Jonsson, 1968) that a reaction occurs between certain




*Fig 7* Adrenergic nerve terminals (*NT*) in the region surrounding nerve fibres (*NF*) entering the organ of Corti Fixed in potassium permanganate *BM* basilar membrane below inner hair cell × 13 800

fixatives such as $KMnO_4$ and several biogenic amines resulting in a precipitate depending upon the concentration of the amine Using routine electron microscopic techniques with glutaraldehyde and or osmium tetroxide as a rule only a few dense-cored vesicles are found in several tissues Incubating the tissue in an amine containing medium or administration of a MAO inhibitor or a 'false' transmitter such as 5 OH DA gives a concentration high enough to produce a distinct precipitate within the vesicles (Hokfelt, 1968, Tranzer & Thoenen 1967) Potassium permanganate has the advantage of demonstrating adrenergic ve




*Fig 8* Adrenergic nerve terminals (*NT*) are seen where myelinated nerve fibres (*NF*) loose their myelin sheaths Potassium permanganate ×31 800

s cles unmistakably but also has the disadvantages of difficult sectioning and poor tissue penetration It is difficult to get a high enough concentration in the modiolus and spiral ganglion to react with the transmitter In the cochlea however where the tissue is thin the reaction between the fixation medium and transmitter is very good Potassium permanganate is a vigorous fixative which destroys certain tissue components giving an inferior morphology compared with that after fixation with glutaraldehyde followed by osmium tetroxide Glutaraldehyde and osmium tetroxide give superior preservation of deep structures due to better penetration In the present study a "false transmitter has been used

A continuous perivascular plexus is found in the modiolus along the vessels in the radiating cochlear nerve bundles and around the spiral vessel in the tympanic lip It is difficult to determine how far from a vessel a terminal can be situated and still exert an effect In investigations on the sympathetic innervation of the intestinal and mesenteric vessels terminals are found at varying distances from the vessels It has been concluded that an effective influence can be exerted at a distance of about 1 $\mu$ (Devine & Simpson 1967) In the cochlea the terminals are also found at varying distances from the vessels No direct contact can be shown between nerve terminals and vessels but the terminals are sometimes found at a distance of only 0 1 $\mu$ from the blood vessel One way in which the sympathetic nervous system may influence sound perception is via an action on the cochlear vascular supply




*Fig. 9.* Adrenergic nerve terminal ($NT_1$) in direct contact with a de(non)-myelinated nerve fibre (NF). Free nerve terminals are seen ($NT_2$). Potassium permanganate. ×35 000.

Apart from the vascular sympathetic innervation an adrenergic innervation independent of blood vessels exists. Using the stretch preparation technique Spoendlin & Lichtensteiger (1967) found a terminal plexus in the habenula region. The density of the fibres was highest at the basal part of the cochlea. With the fluorescence technique Terayama




*Fig 10* Adrenergic nerve terminals among fibroblasts towards the scala tympani at the level of the inner hair cell. Potassium permanganate × 46 800

et al (1965, 1966) found fibres in the habenula region which they interpreted as running from one blood vessel to another With electron microscopy they did not find any adrenergic terminals in this region In the present study adrenergic terminals are found in the spiral limbus between the myelinated nerve fibres and are richly distributed between the nerve fibres just before the demyelinization zone When the demyelinated afferent nerve fibres penetrate the habenula the bundles of axons are surrounded by adrenergic nerve terminals containing dense-cored vesicles Terminals are also situated between the non myelinated dendrites, sometimes in direct contact with nerve fibres Today no method exists however, which makes it possible to show post synaptic membrane specialization Distal to the habenula no sympathetic innervation is found in the organ of Corti or in the stria vascularis In the electron microscope, nerve terminals along non myelinated afferent nerve fibres and the inner spiral bundle contain a large number of empty vesicles and few with a dense core This is a constant finding in osmium tetroxide fixed specimens, whether the animal is pretreated with a 'false transmitter' or not, and after sympathectomy In $KMnO_4$ fixed specimens these dense cored vesicles appear empty or only weakly stained

They are considered to be large granulated vesicles not containing biogenic amines

Apart from this direct innervation of blood vessels and afferent nerve fibres there is a diffuse distribution of adrenergic terminals in the tissue towards the scala tympani and towards the inner sulcus Spoendlin & Lichtensteiger (1967) suggested a sympathetic influence in the habenula region on the threshold for impulse generation in afferent nerve fibres Much work has been done to find out in which way the sympathetic nervous system influences different sense organs In amphibians Hutter & Lowenstein (1955) and Lowenstein (1955) found a reduced threshold for impulse generation in terminals of touch receptors and a facilitated neuromuscular transmission when the sympathetic chain was stimulated

By stimulating the cervical sympathetic ganglion, Beickert et al (1956) showed a decrease in the cochlear microphonic potential Seymour & Tappin (1951) found an initial increase followed by a decrease of microphonics during sympathetic stimulation However, others (Rambo et al, 1953) did not find any effect on cochlear microphonics, either after stimulation or after sympathectomy Russell (1969) when recording the spontaneous afferent nerve impulses from the lateral line organs in *Xenopus* could not discern any change in discharge frequency during stimulation of the sympathetic trunk or after local administration of adrenalin-noradrenalin

Since no adrenergic terminals are found close to hair cells in the organ of Corti the sympathetic influence on cochlear microphonic potentials must be very small or even negligible, which might explain the contradictory results cited above

The presence of many adrenergic terminals *in the habenula region and the short distance* between them and the afferent nerves at the demyelinization zone suggest the possibility of a sympathetic influence on sound perception

## ACKNOWLEDGMENTS

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## ZUSAMMENFASSUNG

Unter Verwendung der elektronenmikroskopischen Technik und Verabreichung einer "falschen" Transmittorsubstanz konnten in der Cochlea adrenerge Nervenfortsätze mit charakteristischen dichten Kernhaltigen Bläschen entdeckt werden Ein kontinuierlicher sympathischer perivaskulärer Plexus wird bis an die Grenze der Habenularegion hin vorgefunden Adrenerge Nervenfortsätze kommen auch unabhängig von Blutgefäßen vor, und diese Innervation ist besonders reichlich in der Habenularegion Nervenfortsätze finden sich häufig unmittelbar vor der *Zone* der Entmyelinisierung und umgeben die afferenten Nervenbündel nach Verlust ihrer Myelinscheide In der Habenula sind Nervenfortsätze zu finden welche direkten Kontakt mit den myelinfreien afferenten Nervenfasern haben Anhäufungen von Nervenfortsätzen zeigen sich im Gewebe in der Nähe des inneren Sulcus und der Scala tympani Keine adrenergen Nervenfortsätze wurden hingegen im Cortischen Organ und in der Stria vascularis gefunden Die morphologische Lokalisation der sympathischen Nervenfortsätze erhärtet ihren Einfluss auf die Schallperzeption entweder durch direkte Wirkung auf die sensorischen Neuronen oder auf dem Wege einer vasomotorischen Steuerung

## REFERENCES

Andrzejewski C 1955 Histologische Studien zur vegetativen und cerebralen Innervation des Innenohres und seiner Gefässe beim Menschen und beim Hund *Z Zellforsch 42* 1

— 1956 Weitere histologische Beobachtungen über die vegetative Innervation des membranösen Labyrinthes *Z Zellforsch 44* 427

Beickert P Gisselsson L & Lofstrom B 1956 Der Einfluss des sympathischen Nervensystems auf das Innenohr *Arch Klin Exp Ohr Nas Kehlkopfheilk 169* 495

Devine C F & Simpson F O 1967 The fine structure of vascular sympathetic neuromuscular contacts in the rat *Amer J Anat 121* 153

Falck B Hillarp N A Thieme G & Torp A 1962 Fluorescence of catecholamines and related compounds condensed with formaldehyde *J Histochem Cytochem 10* 348

Hokfelt T 1968 In vitro studies on central and peripheral monoamine neurons at the ultrastructural level *Z Zellforsch 91* 1

— 1969 Distribution of noradrenalin storing particles in peripheral adrenergic neurons as revealed by electron microscopy *Acta Physiol Scand 76* 427

Hokfelt T & Jonsson G 1968 Studies on reaction and binding of monoamines after fixation and processing for electron microscopy with special references to fixation with potassium permanganate *Histochemie 16* 45

Hutter O F & Lowenstein W R. 1955 Nature of neuromuscular facilitation by sympathetic fibre stimulation in frog *J Physiol* (Lond) *130* 559

Lempert J 1946 Tympanosympathectomy A surgical technic for the relief of tinnitus aurium *Arch Otolaryng* (Chic) *43* 199

Lewis R 1964 The effects of cervical sympathectomy in Meniere s disease *J Laryng 68* 636

Livan M & Del Bo M 1951 De l existence d un plexus nerveux marginal et paramarginal au niveau de la lame spirale et du dillon spiral interne chez l homme *Acta Anat* (Basel) *13* 16

Lowenstein W 1955 Modulation of cutaneous mechanoreceptors by sympathetic stimulation *J Physiol* (Lond) *13?* 40

Lorente de No R 1926 Etudes sur l anatomie et la physiologie du labyrinthe de l oreille et du VIIIe nerf II Quelques données au sujet de l anatomie *des organes sensorielles du labyrinthe Trab Inst Cajal Invest Biol 24* 53

Luft J H 1961 Improvements in epoxy resin embedding methods *J Biophys Biochem Cytol 9* 409

Palumbi G 1954 The innervation of the human inner ear in the light of new histomorphological studies *Sci Med Ital 3* 351

Passe G 1953 Surgery of the sympathetic for Ménieres disease tinnitus and nerve deafness *Arch Otolaryng* (Chic) *57* 257

Rambo J H T Woff D & Freeman G 1953 A research study of the effect of the autonomic nervous system on the internal ear *Ann Otol 62* 1149

Reynolds E 1963 The use of lead citrate at high pH as an electron-opaque stain in electron microscopy *J Cell Biol 17* 208

Richardson K C 1966 Elecron microscopic identification of autonomic nerve endings *Nature ?10* 756

Rhodin J 1954 *Correlation of ultrastructural organization and function in normal and experimentally changed proximal convoluted tubuli cells of the mouse kidney* Stockholm Aktiebolaget Godvil.

Rosen S 1951 Surgery in Menieres disease *Ann Otol 60* 657

Ross M 1971 Fluorescence and electron microscopic observations of the general viscera efferent innervation of the inner ear *Acta Otolaryng* (Stockh) Suppl ?86

Russell I J 1969 *The influence of efferent fibres* on lateral line organs Ph D *Thesis* University of Cambridge

Seymour J C & Tappin J W 1951 The effect of sympathetic stimulation upon the cochlear microphonic potentials *J Laryng 65* 851

Spoendlin H 1966 The organization of the cochlear receptor *Adv Oto Rhino Laryng* (Karger Basel) *13*

— 1971 Degeneration behavior of the cochlear nerve *Arch Klin Exp Ohr Nas Kehlkopfheilk ?00* 27?

Spoendlin H & Lichtensteiger W 1966 The adrenergic innervation of the labyrinth *Acta Otolaryng* (Stockh) *61* 4?3

— 1967 The sympathetic nerve supply to the inner ear *Arch Klin Exp Ohr Nas Kehlkopfheilk 189* 346

Terayama Y Holz E & Beck C 1965 *Fluoreszenz mikroskopischen Nachweis adrenergischen Fasern in der Meerschweinchenschnecke Mschr Ohrenheilk 99* 513

— 1966 Adrenergic innervation of the cochlea *Ann Otol 75* 69

Terayama Y Yamamoto K & Sakamoto T 1968 Electron microscopic observations on the postganglionic sympathetic fibres in the guinea pig cochlea *Ann Otol 77* 1152.

Tranzer J P & Thoenen H 1967 Electron microscopic localization of 5 hydroxydopamine (3 4,5 trihydroxy phenyl-ethylamine) a new false transmitter *Experientia ?3* 743

Watson M L 1958 Staining of tissue sections for electron microscopy with heavy metals *J Biophys Biochem Cytol 4* 475

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# APPLICATION OF IMPEDANCE AUDIOMETRY AS A SCREENING INSTRUMENT

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*Abstract.* More than seven hundred Southeast Alaska pre-school and grade school children were screened with pure tone audiometry and abbreviated acoustic impedance measurements. Impedance data was obtained on 98.8% of the children while audiometric data on only 70.3% due to early age or lack of cooperation. Over 40% of the children failed impedance screening criteria. Of these 70% had Type C tympanograms and 30% had Type B. Abnormal impedance results were found in nearly 10% of the children who passed audiometric screening. Because impedance testing is particularly suitable to the high risk pre-school and early grade school population and because it tests for disease and not just hearing loss, the authors recommend its addition to pure tone testing in screening programs. We would go so far as to say that in younger children if only one were available impedance testing would be preferred.

The primary purpose of a hearing conservation program for children is to identify as many correctable ear conditions as possible in order to prevent the development of hearing loss which might interfere with social and educational growth and development. Conventional pure tone audiometric screening has been assumed to be able to reveal not only children with hearing loss but also those with ear disease which should be referred to medical care. Data from the classical Pittsburgh study of Jordan & Eagles (1961) indicated that audiometric screening, however complete, can not identify all children with ear disease who need medical treatment. In that study over half of 629 otologically abnormal ears had hearing sensitivities equal to or better than zero dB ASA.

Jordan & Eagles (1961) have also shown that many children normally hear at levels below audiometric zero. Thus a child may experience a significant decrease in hearing sensitivity without this decrease being detected by the usual audiometric screening.

In our early experience with pure tone audiometric screening in the schools of Southeast Alaska we noticed that many individuals who had "passed" the screening at a level 25 dB re ANSI were identified by an examining physician or otologist to have a middle ear disease which required medical or surgical treatment. It became evident that new screening techniques were needed to properly identify these children.

It was the purpose of this study to evaluate the use of acoustic impedance measurements in conjunction with pure tone screening as a means of identifying children with middle ear disease needing treatment. While acoustic impedance measurements have been made for years, their utilization as a screening device to detect middle ear disease has been a relatively late development. Brooks (1969) tested over 1 000 five to ten year old English school children by this means and found it a simple

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and effective means of detecting middle ear fluid According to his data, the incidence of middle ear fluid decreased as age increased

## SUBJECTS

The study population consisted of 710 children enrolled in 10 pre schools and four elementary schools in Southeast Alaska They were grouped for the study into three groups pre schoolers, grades kindergarten through three, grades four through twelve Pre school children were five years old or younger, children in kindergarten through third grade ranged from ages five through nine, and children in fourth through twelfth grade were 9 years of age or older

## METHODS

To insure an unselected population, all children in each of the 14 schools were screened utilizing both pure tone and acoustic impedance measurements Pure tone screening was accomplished by using a Maico 2A portable audiometer, and acoustic impedance measurements were accomplished by using either the Madsen Z0 70 or Z0-72 electro-acoustic impedance meters Both the Maico audiometer and the acoustic impedance meters were calibrated using a Bruel & Kjer sound level meter prior to each school screening

The pure tone screening consisted of checking for patient's response to pure tones of 500, 1 000, 2 000 and 4 000 Hz at a level of 25 dB re ANSI This level of presentation was chosen because the tests all had to be administered in an empty classroom without sound treatment and ambient noise was higher than desirable

Acoustic impedance measurements consisted of the following

1 Inserting the probe tip and obtaining an air tight seal
2 Introducing a positive pressure of 200 mm H O into the ear canal and then checking the seal by monitoring the manometer
3 Taking the reading in acoustic ohms at this pressure reading of −200 mm
4 Reducing the pressure until the peak compliance of the tympanic membrane was noted by the swing of the balance meter from the left to the right
5 Noting the tympanogram type by ascertaining the middle-ear pressure reading at that point
6 Taking the reading in acoustic ohms at this level of peak compliance
7 At the most compliant position presenting to the contralateral ear a 1–2 second 100 dB HTL pure tone stimulus of 1 000 Hz, and noting the presence or absence of the acoustic reflex as indicated by the deflection of the balance meter
8 Calculating the static acoustic impedance from the two previous acoustic ohms readings by the use of a Madsen nomogram
9 Repeating the process on the other ear

The criteria for failure of pure tone screening consisted of failure to respond to tones presented at 25 dB ANSI at frequencies of 500, 1 000, 2 000 or 4 000 Hz in either or both ears

Three *criteria for failure of impedance* screening were chosen on the basis of previous experience

1 A Type A tympanogram with an absent acoustic reflex
2 A Type B tympanogram
3 A *Type C tympanogram*

The static impedance values were not used as a criterion for failure of acoustic impedance screening because of the frequent overlap of normal and abnormal values in many disease states

Jerger's classification of tympanograms into Types A B and C was used for simplicity of description A Type A tympanogram exhibits maximum tympanic membrane compliance when the pressure in the external auditory canal is near zero The pressure at this most compliant point may be slightly negative but

does not equal or exceed −100 mm H O A Type B tympanogram exhibits little or no compliance change over wide pressure variations in the external ear canal A Type C tympanogram has a compliance maximum in the negative pressure range between −100 and −400 mm H O

## RESULTS

Of the 710 children in the study, audiometric pure tone screening results could not be obtained on 29 7% of the children because of the early age of the child (35 were under the age of two) or because of the child s lack of cooperation (Table I)

It was possible to obtain acoustic impedance results on all but 1 3% of the children providing results on 1 401 ears (Table II) Of these 41 5% failed our criteria for acoustic impedance screening Of the total 13 0% had Type B tympanograms and 28.5% had Type C tympanograms There were no instances of Type A tympanograms with absence of the acoustic reflex although we have seen this in older children adults and some young children not included in this study (By our testing method the acoustic reflex was always absent in ears with Type B tympanograms but remained elicitable in one third of those ears with Type C tympanograms) The highest percentage of abnormalities was seen in pre school and young school age children with the lowest percentage found for the fourth through twelfth grade group In the kindergarten through third grade group just over 50% had either Type B or Type C tympanograms

Of those who could be tested by pure tone screening 9 7% had "normal hearing" but

Table I *Number of patients unable to be audiometrically screened*

| | |
|---|---|
| Pre school | 121 |
| Kind 3rd | 26 |
| 4th 12th | 0 |
| Total | 147 |

Table II *Results of impedance screening*

| | A | B | C |
|---|---|---|---|
| Pre school | | | |
| R ear | 239 | 58 | 116 |
| L ear | 240 | 49 | 124 |
| Kind 3rd grade | | | |
| R ear | 77 | 23 | 57 |
| L ear | 77 | 23 | 56 |
| 4th 12th grade | | | |
| R ear | 96 | 13 | 22 |
| L ear | 93 | 17 | 21 |
| Total | 822 | 183 | 396 |
| % of total ears | 58 5 | 13 0 | 28 5 |
| Total[a] patients | *710* | | |
| Total ears | *1 401* | | |

[a] No results could be obtained on 9 patients or 1.3% of total seen

failed the acoustic impedance screening (Table III) With one exception these were all in the pre school and kindergarten through third grade groups This 9 7% represents children who but for the acoustic impedance results would not be referred for appropriate medical attention

## DISCUSSION

The incidence and magnitude of middle ear disease in Alaska is staggering The cohort study of Reed et al (1967) showed that nearly two of every three Eskimo babies had experienced purulent otorrhea from acute suppurative otitis media by the age of 2 years Harker & Van Wagoner (1972) in a 3 month period of observation found 15% of a mixed racial Southeast Alaska grade school population had either middle ear effusion or persistent abnormal negative intratympanic pressure indicative of eustachian tube malfunction A mixed racial survey by Hayman &

Table III *Number of children with normal hearing failing impedance screening*

| | |
|---|---|
| Pre school | 56 |
| Kind 3rd | 10 |
| 4th 12th | 1 |
| Total | 67 |
| % of total screened | 9 7% |

Kester (1956) revealed 53% of 900 children had perforation, retraction, scarring, or inflammation of the tympanic membrane Our high percentage of abnormal test of middle ear function thus did not surprise us

Seventy percent of ears failing our impedance screening criteria had Type C tympanograms which reflect negative intratympanic air pressure This occurs because the eustachian tube fails to open during swallowing The air in the tympanic cavity, which is constantly being absorbed via mucosal capillaries is no longer replenished, and a partial vacuum results The causes of this eustachian tube malfunction are many some are short-lived and recovery may occur without treatment, but others may never resolve without medical or surgical treatment Ideally all these patients should be seen by an otologist

Thirty percent of the ears failing the impedance screening exhibited Type B tympanograms with a flat tympanic membrane compliance function If the tympanic membrane was intact and the child had no previous surgery on the test ear, middle ear fluid was nearly always present, although its viscosity was sometimes thick and sometimes thin Otological referral and treatment is always indicated with a flat compliance curve It was extremely rare in our experience for a Type B tympanogram to revert to a Type C, but the opposite was common and represented progression of disease

Some pre school children can be rapidly conditioned to respond to pure tones and a quick valid screening can be obtained More often it is time consuming and considerable experience and skill are required to obtain valid results Sometimes it is just impossible It was encouraging to get meaningful data on middle ear function by impedance testing in such a high percentage of these children The pre school and early school grades had the highest incidence of impedance screening failures and it was almost exclusively in these younger groups where some children passed audiometric screening but exhibited abnormal impedance measurements Impedance screening represents an effective means of increasing the detection rate of middle ear disease needing otologic referral in these younger children The goal of screening should be the detection of disease, not just the detection of those diseases which at the time of the test are accompanied by hearing loss

## ZUSAMMENFASSUNG

In der vorliegenden Studie wurden uber 700 Vorschul und Grundschulkinder in Sudost Alaska mit Tonaudiometrie und einem vereinfachten Impedanzmessverfahren im Screeningtest getestet Impedanzdaten konnten von 98 8% der Kinder aufgenommen werden wahrend dagegen audiometrische Daten nur von 70 3% erhalten werden konnten weil die Kinder zu jung waren oder nicht entsprechend mitarbeiteten Bei uber 40% der Kinder fielen die Impedanzdaten ausserhalb des Rahmens der Impedanzkriterien im Screeningtest Von dieser Gruppe hatten 70% Tympanogramme vom Typ C und 30% vom Typ B Anormale Impedanzwerte wurden bei nahezu 10% der Kinder bei denen audiometrisches Screening durchgefuhrt werden konnte festgestellt Da sich der Impedanztest in Risikofallen bei Vorschulkindern und jungeren Grundschuljahrgangen als gut anwendbar erwiesen hat und da er gewissermassen die Basis der Schadigung und nicht den Horverlust als solchen testet schlagen die Autoren diesen als Zusatztest zu den tonaudiometrischen Tests im Screeningprogramm vor Sollte bei jungeren Kindern nur ein Test durchfuhrbar sein so ist nach Ansicht der Autoren dem Impedanztest der Vorzug zu geben

## REFERENCES

Eagles E L 1972 Selected findings from the Pittsburgh study *Trans Amer Acad Ophthal Otolaryng 76* 343

Harker L A & Van Wagoner R 1972 Unpublished data

Hayman C R & Kester F E 1956 Eye ear nose and throat infection in natives of Alaska *Northwest Med 56* 423

Jordan R E & Eagles E L 1961 The relation of air conduction audiometry to otologic abnormalities *Ann Otol 70* 819

Reed D Struve S & Maynard J E 1967 Otitis media and hearing deficiency among Eskimo children A cohort study *Amer J Public Health 57* 1657

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# A COMPARATIVE STUDY OF THE DEVELOPMENT OF HEARING AND VISION IN VARIOUS SPECIES COMMONLY USED IN EXPERIMENTS

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*Abstract* Inception of hearing and opening of eyelids were examined in cat rabbit dog and mink The hearing tests were performed with pure tones and bands of noise observing various types of behavioural responses Hearing was found to occur at an average age of 5 days in cat 7 days in rabbit 14 days in dog and 29 days in mink The frequencies first responded to and the successive development of hearing for other frequencies are reported and related to data from the literature for five other species Opening of eyelids started at an average age of 7 days in cat, 10 days in rabbit, 14 days in dog and 33 days in mink The possible connection between blood supply to the various coils of the cochlea (in man) and the ontogenetic development of hearing and the deterioration of hearing with aging and acoustic trauma is discussed

The age at which hearing and vision occur in different species has been the subject of several publications *hearing* (Wada, 1923, Larsell et al, 1935, 1944, Rose et al, 1957, Marty, 1962, Alford & Ruben, 1963, Anggård 1965, Mikaelian & Ruben 1965, Crowley & Hepp-Reymond 1966, Hilding et al, 1967, Pujol et al, 1967, Pujol & Marty, 1968, Flottorp & Foss, 1970 Foss & Flottorp, 1970, Sugiura & Hilding 1970, Johansson, 1972, Johansson et al 1972) *vision* (Contino, 1907, Jasper et al, 1937, McCrady 1938, Hunt & Goldring, 1951, Marty 1962, Rose, 1968, Rose & Ellingson, 1968 Erichsen, 1969)

Rarely have the authors tried to correlate their observations about the start of functioning of one sensory modality with another, or with other characteristics, such as weight of the animal The age of an animal may not be the only or best parameter referred to in such studies At least as much information is obtained when age is correlated with body weight

Some investigators have been concerned with the frequency range to which the animal has first responded (Larsell et al, 1935, 1944, McCrady et al, 1937, 1940, Mikaelian & Ruben, 1965, Änggård, 1965, Crowley & Hepp-Reymond, 1966, Pujol et al, 1967) However, this problem has most often been neglected The investigators have shown little interest for correlating their observations of the development of response in the sense organ with the blood supply to the sense organ

The object of the present study was to determine when hearing and vision occur in various species and to correlate these data with the animals' weight We have also tried to determine the frequency range first responded to In addition we discuss our findings with respect to the representation of frequencies along the basilar membrane and to the blood supply of the inner ear

## MATERIAL AND METHODS

We have examined four different species cat, rabbit dog and mink, using animals from dif

Table I *Number of animals at various ages submitted to hearing test*

| Species | Litter no | Litter size | Animals tested: No | Sex ♂ | Sex ♀ | Age (in days) when submitted to hearing test: 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 20 | 22 | 24 | No of hearing tests |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Cat | 1 | 2 | 2 | 1 | 1 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | | | | | | | | | | | | | 14 |
| | 2 | 1 | 1 | | 1 | | | | | | | 1 | 1 | 1 | 1 | 1 | | | | | | | | | 5 |
| | 3 | 4 | 4 | 3 | 1 | | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | | | | | | | | | | | 32 |
| | 4 | 4 | 4 | 2 | 2 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | | | | | | | | | 44 |
| Rabbit | 1 | 7 | 2 | 1 | 1 | | | | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | | | | | | | | | 16 |
| | 2 | 7 | 2 | 1 | 1 | | | | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | | | | | | | | | 16 |
| | 3 | 6 | 2 | 1 | 1 | | | | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | | | | | | | | | 16 |
| Dog | 1 | 6 | 5 | 3 | 2 | | | | | | | | | | | | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 40 |

| Species | Litter no | Litter size | No | ♂ | ♀ | Age (in days) when submitted to hearing test: 26 | 27 | 28 | 29 | 30 | 31 | 32 | 33 | 34 | 35 | 36 | 37 | 38 | 39 | 40 | 41 | 42 | 43 | No of hearing tests |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Mink | 1 | 4 | 2 | | 2 | | | | 1 | 1 | 1 | 1 | 1 | | 1 | 1 | 1 | 1 | 1 | 1 | | 1 | 1 | 13 |
| | 2 | 2 | 2 | 1 | 1 | | | 1 | 1 | 1 | 1 | 1 | | 1 | 1 | 1 | 1 | 1 | 1 | | 1 | 1 | | 13 |
| | 3 | 2 | 2 | 1 | 1 | 1 | 1 | 1 | 1 | | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 2 | | | 16 |

ferent litters—in cat, four litters, in rabbit and mink, three litters, and only one litter in dog (see Tables I and II)

Our cats are of the usual mixed breeds—house cat (*Felis catus*)—with rather substantial variation in number of kittens in each of the four litters, and gestation length unknown, although the birth date is known None of the kittens were white (suggestive of hereditary deafness)

The rabbits are well defined, being a New Zealand white (albino with red eyes), gestation length of all three litters being precisely 31 days, equal to reported average length (Erichsen, 1969), and litter size (6 7) being very close to reported average of 7 (Erichsen, 1969)

Table II *Number of animals tested and number of hearing tests performed*

| Species | No of animals tested | No of hearing tests |
|---|---|---|
| Cat | 11 | 95 |
| Rabbit | 6 | 48 |
| Dog | 5 | 40 |
| Mink | 6 | 42 |
| Total | 28 | 225 |

The mink is the dark mink (standard) Gestation length was 46 3 days on average This was of little importance, however, since the development of the fetus varies substantially from mother to mother The average gestation length in standard mink is 49 1 days (Udris, 1970) The average size of our litters was 2 7, somewhat smaller than reported by Udris (1970) 5 2

The dog is the Norwegian Dunkerhound, the gestation length 64 days, close to the reported average 63 days (Erichsen, 1969) Litter size was 6, very near to the average size for Dunkerhound 6 9 (Lyngset & Lyngset, 1970) Only puppies of black and merle colour were used in the present work, excluding a white puppy (which forms part of a study of hereditary deaf animals to be published later)

The hearing tests have been performed in an approximately anechoic sound proof chamber, using a Peter's Audiometer, type AP 6 together with a Peter's Power Amplifier and Loudspeaker, type AS 11

The animal was placed in front of the loud speaker either on a resilient pad or held in the hands of the experimenter (see Fig 1 for the test situation) The younger the animal, the more necessary it was found to let it feel the warmth of the hands of the experimenter, in order that it was sufficiently relaxed for the test

Pure tones (125–8 000 Hz in octave steps, plus 750 Hz), and narrow band of noise centered at 750 Hz (approximately 1/3 octave) and "white" noise (limited by the loudspeaker's frequency response) were used as test stimuli at intensities shown in Table III

A positive response of the animal to the signal was judged to be either a startle of varying intensity, a pinna or cochleo-orbicular reflex or some type of "behaviour change" evaluated according to strength, as presented in Table III The test was rather time-consuming as it was necessary to observe the animal very carefully as to the kind of "status" it had before applying the stimulus By experience we learned that for instance no sound production from the animal could be accepted during the testing period The stimulus had to be repeated sufficiently often in order to ensure the validity of the response Both authors served as observers one of us controlling the apparatus (stimulus) the other one keeping the animal in correct position

In order to perform a thorough hearing test, we found it necessary to limit the number of animals to about 6 on each test day In another study we manage to test 12 animals each day However, this was found to be rather tiring for both experimenters and animals Of the 20 rabbits from 3 litters we picked out one of each sex at random from each litter All tests were performed during the morning hours observation of the eye lids and of the weight after finishing the hearing test

Ophthalmoscopy was used in dog and mink in order to examine whether opening of the eyelids corresponded with the ability to see, i e presence of the orbicular reflex (contraction of m orbicularis oculi) and of pupil variation (contraction of m sphincter pupillae) as a response to light In observing the status of the eyelids we employed a three category scale



*Fig 1* Animal (cat) under test in front of loud speaker

1 Beginning When a fissure could just be observed, always starting at the medial angulus
3 Full Opening completed both laterally and vertically
2 Half The stages in between 1 and 3

The weight of the animals was measured in grams, using a Mettler Precision Balance model P 1200

## RESULTS

The results of the hearing tests of one animal are shown in Table III (cat), and for all

Table III *Result of hearing test of one animal (cat)*

| Test stimulus | | Age (days) 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| White noise (108 dB SPL) | | − | − | − | − | − | − | − | + − | − + | − + + |
| Noise band 750 Hz (113 dB SPL) | | − | − | − | − | − | (+) | + + | + + | + − | + + + |
| **Pure tone audiometry** | | | | | | | | | | | |
| Frequency (Hz) | Sound level (dB SPL) | | | | | | | | | | |
| 125 | 120 | − | − | − | − | − | (+) | + + | − | − + | + − |
| 250 | 120 | − | − | − | − | − | + + | (+) | + + | + + | − + |
| 500 | 118 | | − | − | − | − | − | + − | − + + | + + | − − − |
| 750 | 115 | − | − | − | − | − | − | − | + | − + | + + − |
| 1 000 | 108 | − | − | − | − | − | − | − | − | + + | + + |
| 2 000 | 103 | − | − | − | | − | − | − | − | + − | − + + |
| 4 000 | 106 | − | − | − | | − | − | − | (+) | + | + − |
| 8 000 | 105 | − | − | − | | − | − | − | − | (−) | + |

\+ + + Extremely strong startle reflex
\+ + Easily recognized startle reflex or pronounced pinna reflex
\+ Less pronounced startle, pinna, cochleo-orbicular reflex or ' behaviour change
(+) Uncertain response
− No response

animals in Table IV We have calculated the average age in days when response was first observed to the various types of stimulus used The last column in Table IV shows the average age in days when the first hearing response was observed, irrespective of the type of the stimulus used The first species to hear was cat, thereafter rabbit, dog and finally mink

Results of ophthalmoscopy justify the con-

Table IV *Result of hearing test of all animals*

| Species | | Frequency of pure tones (Hz) 125 | 250 | 500 | 750 | 1 000 | 2 000 | 4 000 | 8 000 | Type of noise: Noise band 750 Hz | White noise | Stimulus producing the first hearing response |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Cat | Ā | 6.2 | 6.3 | 6.5 | 6.5 | 7.2 | 7.5 | 8.2 | 9.1 | 6.3 | 6.9 | 5.4 |
| | s | 2.1 | 1.4 | 1.4 | 1.4 | 1.3 | 1.1 | 1.1 | 1.3 | 1.2 | 1.5 | 1.9 |
| | n | 11 | 11 | 11 | 11 | 11 | 11 | 11 | 11 | 11 | 11 | 11 |
| Rabbit | Ā | 7.7 | 7.0 | 6.8 | 6.7 | 8.5 | 8.3 | 9.2 | 9.7 | 6.8 | 7.7 | 6.7 |
| | s | 1.0 | 0.9 | 0.8 | 0.5 | 1.1 | 0.5 | 1.0 | 1.0 | 0.4 | 0.8 | 0.5 |
| | n | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 |
| Dog | Ā | | 15.3 | 14.8 | 14.5 | 16.2 | 18.8 | 17.6 | 21.5 | 14.6 | 13.8 | 13.8 |
| | s | | 0.6 | 0.5 | 1.3 | 2.5 | 4.8 | 4.2 | 1.0 | 0.9 | 0.8 | 0.8 |
| | n | | 3 | 4 | 4 | 5 | 5 | 5 | 4 | 5 | 5 | 5 |
| Mink | Ā | | 34.3 | 30.0 | 30.2 | 34.0 | 35.0 | 38.5 | | 31.5 | 31.2 | 29.2 |
| | s | | 4.5 | 2.8 | 2.6 | 3.1 | 5.0 | 6.4 | | 1.4 | 1.5 | 2.3 |
| | n | | 6 | 6 | 6 | 6 | 4 | 2 | | 6 | 6 | 6 |

Ā − Average age in days when response was first observed
s − Standard deviation of A
n − Number of animals

clusion beginning of opening of eyelids corresponds with inception of visual function The pupil variation was, however, far less pronounced than the orbicular reflex

Fig 2 shows the correlation in occurrence of hearing and vision in the four test species In Fig 3 the development of hearing in different frequency ranges is presented, together with opening of eyelids, as a function of age in the four test species Weight as a function of age, together with the first observed auditive response and occurrence of vision in the four test species, are shown in Fig 4

In Table V we have presented our results together with those reported by other investigators, in order to review the development of hearing and vision in various species as a function of age For the same species a review is presented of the localization of frequencies along the basilar membrane, together with the frequency range in which the first auditive response was observed (see Fig 5)

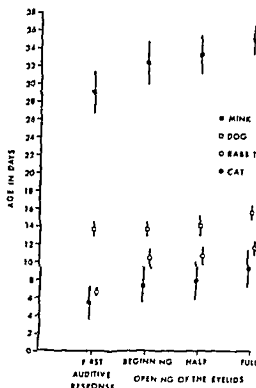



*Fig 2* Age (arithmetic means) when first audi response and when opening of the eyelids in the species were observed Vertical lines indicate sta ard deviation (one sigma to each side)

## DISCUSSION

*Inception of hearing versus vision*

In the four species studied by us it seems as if hearing occurs a little earlier than the opening of the eyelids This is in general agreement with reports of similar work with other species (Table V) The only exception to t rule is the guinea pig The data for this speci however, is probably among the more unce tain, since opening of the eyelids is observ before birth As a general rule it may

Table V *Review of the development of hearing and vision in various species as a function of age*

| Species | Hearing development Behavioural response | | Opening of the eyelids | |
|---|---|---|---|---|
| | Age | Reference | Age | Reference |
| Man (*Homo sapiens*) | 24 weeks[a] | Johansson et al (1972) | 24 weeks[a] | Contino (1907) |
| Guinea pig (*Cavia porcellus*) | 5-6 hours | Wada (1923) | 48-56 days[b] | Jasper et al (1937 |
| Cat (*Felis catus*) | 5 days | Foss & Flottorp | 7 days | Foss & Flottorp |
| Rabbit (*Oryctolagus cuniculus*) | 7 days | Foss & Flottorp | 10 days | Foss & Flottorp |
| Mouse (*Mus musculus*) | 10 days | Mikaelian & Ruben (1965) | 12 14 days | Erichsen (1969) |
| Rat (*Rattus norvegicus*) | 10 days | Wada (1923) | 12 14 days | Erichsen (1969) |
| Dog (*Canis familiaris*) | 14 days | Foss & Flottorp | 14 days | Foss & Flottorp |
| Mink (*Mustela vison*) | 29 days | Foss & Flottorp | 33 days | Foss & Flottorp |
| Opossum (*Didelphis virginiana*) | 50 days | Larsell et al (1944) | 50-60 days | McCrady (1938) |

[a] Fetal age therefore hearing and vision 16 weeks before birth
[b] Fetal age therefore vision 7 15 days before birth assuming a gestational period of 63 days (Erichsen 1969)

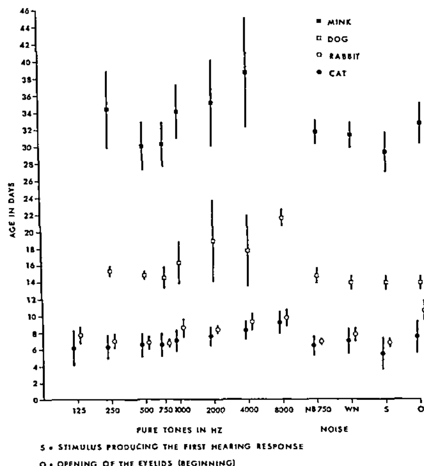



*Fig 3* Hearing and vision (arithmetic means) in test species as a function of age Vertical lines indicate standard deviation (one sigma to each side)

concluded that if one observes open eyes, i e full opening of the eyelids, the animal should hear, otherwise something is probably wrong with the hearing organ

In addition to the method used by us (observation of the behavioural responses to sound), the function of a sense organ may be studied by recording the neural activity at various levels i e hearing by means of cochlear microphonics, action potentials or cortical responses, and vision in a similar manner (action potentials, cortical responses) It is conceivable that electrophysiological responses may be obtained before it is possible to observe behavioural responses Based upon observations of several animals, however, we have reason to believe that the reflex mechanisms we have utilized were mature before the response to sound stimulation was observed Our method does not, however, allow a subtle evaluation of the maturation of hearing The criterion used some type of behaviour reflex involving the motor system cannot elucidate the hearing function for stimulus intensities below the reflex threshold—which is known to be rather high The electrophysiological method should, therefore, be more sensitive and provide results indicating occurrence of hearing a little earlier than we have observed in the same species The difference seems to be a constant factor of 2 days for hearing, and 2–3 days for vision (cat, rabbit, mouse, rat, opossum)

The middle ear may, however, lag in development In mink we could observe auditive response before the middle ear was fully developed In some electrophysiological studies the sound stimulus more or less bypasses the mid-

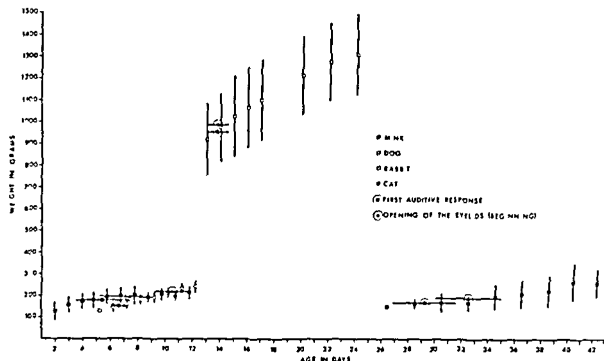


*Fig 4* Weight (arithmetic means) as a function of age, together with first observed auditive response and vision (arithmetic means) in cat, rabbit dog and mink Vertical and horizontal lines indicate standard deviation (one sigma to each side)

dle ear, thus eliminating a possible attenuating factor of the unmatured middle ear

The possible selective influence of an unmatured middle ear upon the transmission in different frequency bands has not been studied by us We know, however, from clinical experience on humans that atresia of the ear canal (maximum loss 50–70 dB), fluid in the middle ear (maximum loss 30–40 dB) and similar middle ear pathology usually attenuate the entire auditive frequency range We therefore do not consider that a possible lag in proper functioning of the middle ear should be responsible for a substantial delay of an observable auditive response to the sound intensities used

In comparing our results with those reported in the literature it is also necessary to keep in mind the uncertainty about gestation length (date of conception, maturity at parturition), number of offspring per litter, possible differences between different breeds and between different animal husbandries as to feeding and care

In addition there may be differences in definition of occurrence of hearing and vision We have, for instance, defined our observations of opening of the eyelids *in three* categories and chosen "first observed opening" as our criterion for vision, whereas most researchers report nothing about their criteria For hearing, the type of stimulus used is of great importance, since our findings show that hearing starts earlier for certain stimulus frequencies than for others

In the following we will discuss in some detail results for our four examined species

*Rabbit*

Of our animals the rabbit is the best defined Our observations show that hearing occurs at the age of 7 days (Table IV) We have not been able to find any corresponding data in the literature, though Anggård (1965) concludes from an electrophysiological study that hearing occurs at the age of 5 days *This is* in good agreement with the earlier mentioned difference between results obtained using be

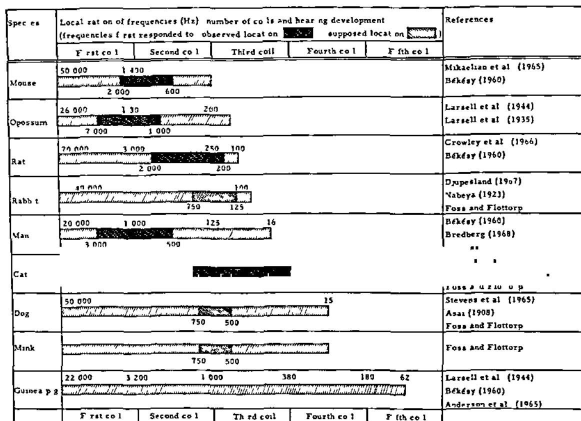



*Fig 5* Review of the localization of frequencies along the basilar membrane in various species together with the frequency range in which the first auditive response was observed The bars indicating the basilar membranes are schematically drawn, giving each coil the same length Therefore the figure has no resemblance to the physical dimensions

havioural and electrophysiological responses

*Opening of the eyelids, according to our* definition and observation, occurs at the age of 10 days Marty (1962) reported 9 days, and Erichsen (1969) 9–10 days

Our observation of weight and its daily increase agrees with Venge (1953)

*Cat*

Our cats are of the usual mixed breeds with rather substantial variations in number of kittens in each of the four litters, and gestation length unknown We observed rather large differences in age at time of occurrence of hearing and vision in the different litters This was, however, not unexpected Litter number one consisted of only 2 kittens, with signs of overmaturity (at the age of 2 days *weight 185 grams and hearing present, vision* at the age of 4 days) According to Hall & Pierce (1934) the average litter size is 3 9, whereas the corresponding figure for our litters is 2 8 Our two litters of normal size (4) dominate the presented data average age at inception of hearing being 5 days and for vision 7 days Pujol et al (1967) and Pujol & Marty (1968) reported 2–3 days for hearing Their results are, however, based upon electrophysiological measurements and fit therefore very well in the earlier-mentioned scheme of 2 days difference between electrophysiological and behavioural responses

Marty (1962) and Erichsen (1969) report later occurrence of vision (9 days and 10 days)

than we do The reason for this discrepancy may be different definitions of opening, or may be due to different sizes of litters and uncertainty of gestation period

Our observation of weight and its daily increase agrees with Hall & Pierce (1934)

*Dog*

We examined 5 puppies and found that hearing occurred at an average of 14 days, coinciding with occurrence of vision Based upon a study of 10 different breeds, Parry (1953) reports opening of eyelids at an age of 10–15 days, Erichsen (1969) 10–14 days. We have not been able to trace any corresponding hearing data in the literature We are, however, convinced that a greater number of litters would provide results indicating the same small difference between occurrence of hearing and vision as reported for the other species Our dogs had rectilinear weight increase (36 grams per day) equivalent to results for beagle (38 grams per day) (Andersen, 1970)

*Mink*

We conclude from our observations of 6 minks from three different litters that hearing occurs at an average age of 29 days, vision 4 days later The age observed by us is distinctly greater than reported by Hilding et al (1967) 2 weeks and by Sugiura & Hilding (1970) 3 weeks The difference between results may be due to the earlier mentioned variation in "real" gestation length, although different methods probably are the most important reason The standard deviation for our mink data is rather great We believe, however, they are reliable because we have corresponding results from examination of the white Hedlund mink This species was tested in greater number of litters and animals (Flottorp & Foss, 1970, Foss & Flottorp, 1970, further details in a following publication)

As can be seen in Table V, we have observed opening of eyelids at an average age of 33 days (4 days later than inception of hearing) We have not been able to find corresponding information in the literature The reported age, resulting from our rather small number of animals, is exactly the same as observed by us in the just mentioned examination of Hedlund mink, comprising 18 kits Our observation of weight and its daily increase agrees with results obtained at The Agricultural University of Norway during a period of 15 years from 3–4 000 mink kits (Rimeslåtten, 1973)

Table VI *Cochlear localization (human) for tones based on data from Bredberg (1968) and Békésy (1960)*

| Coil | Distance from the basal end of the cochlea: Angular measure (degrees) | Distance from the basal end of the cochlea: Linear measure (mm) | Frequency (Hz) | Coil |
|---|---|---|---|---|
| | 0 | 0 | (20 000) | |
| | 15 | 3 | 10 000 | |
| | 35 | 5 | *8 000* | |
| | 60 | 7 | 6 500 | |
| | 120 | 10.5 | 4 200 | |
| | 135 | 11 | *4 000* | |
| 1st | 170 | 13 | 3 000 | 1st |
| | 180 | 13.5 | *2 800* | |
| | 215 | 15 | 2 400 | |
| | 255 | 16.5 | *2 000* | |
| | 270 | 17 | 1 800 | |
| | 360 | 21 | *1 000* | |
| | 425 | 23 | 750 | |
| | 505 | 25 | *500* | |
| | 540 | 26 | 430 | |
| 2nd | 560 | 26.5 | 400 | 2nd |
| | 600 | 27.5 | *250* | |
| | 625 | 28 | 200 | |
| | 720 | 30 | *125* | |
| | 745 | 30.5 | 100 | |
| 3rd | 875 | 32.5 | 50 | 3rd |
| | 1 030 | 34 | (16) | |

*Frequency localization and maturity of hearing*

Although our sound stimuli, from the weakest to the strongest, vary 15 dB in SPL, we consider them to be adequate for determining the frequency range first responded to We gave the lower frequencies, for which the ear is usually a little less sensitive, the highest sound pressure level The sound pressure level for all stimuli was very high, approaching

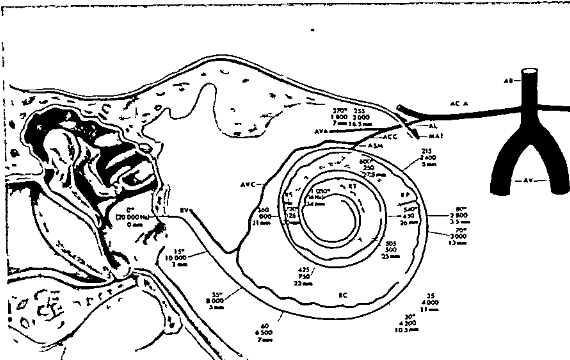



*Fig 6* Human cochlea (anterior view of the right cochlea) blood supply and localization of frequencies Schematically drawn from data extracted from Bredberg (1968) Bekésy (1960) Eichler (1892) Siebenmann (1894) Nabeya (1923) Scuderi & del Bo (1952) Charachon (1961) and Axelsson (1968) *AV* Arteria vertebralis *AB* Arteria basilaris *ACIA* Arteria cerebelli inferior anterior *AL* Arteria labyrinthi *AVA* Arteria vestibuli anterior *ACC* Arteria cochleae communis *AVC* Arteria vestibulo-cochlearis *ASM* Arteria spiralis modioli *RV* Ramus vestibularis (of AVC) *RC* Ramus cochlearis (of AVC) *RP* Ramus primus (of ASM) *RS* Ramus secundus (of ASM) *RT* Ramus tertius (of ASM) *MAI* Meatus acusticus internus

the pain limit (we used ear defenders during the test period)

According to Boothroyd & Cawkwell (1970) the possibility of tactile response to loud sounds below 500 Hz should be considered Our animals however, did not respond to these low tones at an age for which the tactile sense without doubt was mature In addition we have never observed the deaf Hedlund mink to respond to these low tones (study to be published later) The reported response to low frequency sounds in this investigation should therefore be genuine auditive

The frequency range first responded to is in all four species located to the second and third coil of the cochlea (see Fig 5) We consider this to reflect the status of development of the cochlea Although our method is rather coarse—only examining response to very high stimulus intensity—we find no reason to believe that the development of hearing shows any discontinuity Thus the first observed response to high stimulus intensity in a certain frequency range only is considered to be indicative for the inception of hearing in the corresponding tonotopic location of the cochlea

The retrocochlear part of the afferent system in particular the auditory cortex is probably a little ahead of the cochlea in maturation (Larsell et al, 1935, Marty, 1962 Chaloupka et al, 1968 Pujol & Marty, 1968) Therefore our study of the cochlear tonotopic localization should not be concealed by this part of the reflex arc The middle ear may lag in maturity

as mentioned earlier, however, should not cause a significant change in response to different frequencies

*It appears from data of other researchers* that in the second and third coil of the cochlea—for some species also in the range between first and second coil—the organ of hearing first develops ability to respond to sound (for references see Fig 5) So far we can find no support for the oft advocated statement that hearing starts at the basal coil (see, e g Pujol et al, 1967)

The very interesting problem about a possible correlation between the tonotopic localization of frequencies first responded to and the topography of the blood supply to the cochlea, has been at the focus of our interest Our working hypothesis has been that the partition of the cochlea having the best blood supply will develop first and will be first matured to respond to acoustic stimulation

We realize that the ontogenetic development is not by chance governed by the presence or absence of sufficient blood supply Probably the pattern of development is genetically guided, however, the goal is reached by a careful *layout of the vascular system, ensuring the* organs and/or parts of organs wanted to be ahead in the development of the best blood supply

Since the most valid available data about the localization of frequencies along the basilar membrane and of the topography of the blood supply is for the human ear, we have tried to accumulate these data from different sources and combine them (see Table VI and Fig 6) From observations on the human fetus, Johansson (1972) suggests that the development of the cochlea starts around 1 500 Hz and proceeds in both directions This conception fits very well with the topography of the blood supply to the cochlea (Fig 6)

The first branch (ramus primus, RP) of A spiralis modioli (ASM) is rather thin, whereas the second branch (ramus secundus, RS) is distinctly thicker (Siebenmann, 1894, Nabeya 1923) Therefore, the upper quart of the first coil, together with the lower half of the second coil, i e the range from about 2 000 Hz to 500 Hz, should have the best blood supply

*On the other hand,* the vestibular branch (ramus vestibularis, RV), although a rather thick branch, primarily destined for supplying the vestibular apparatus, gives off only tiny twigs to the basal area of the cochlea corresponding to frequencies above 9 000 Hz (Axelsson, 1968) Especially the extreme basal end of the cochlea (caecum vestibulare) has very poor blood supply, being vascularized from a tiny recurrent branch (a radial section of stria vascularis contains only one capillary) (Axelsson, 1968) This fact may account for the observed vulnerability of hearing above 12 000 Hz in man (Flottorp, 1973)

Anastomosis exists between A vestibulocochlearis (AVC) and A spiralis modioli (ASM) and also between the three branches (RP, RS and RT) of A spiralis modioli (Axelsson, 1968) This does not appear from the drawing in Fig 6, because we intended to show distinctly which parts of the cochlea were supplied from the various branches The anastomosis between AVC and ASM (at 4 000 *Hz) is poorly developed* (Siebenmann, 1894, Nabeya, 1923, Axelsson, 1968) Clinical experience has for many years drawn attention to presbyacusis and acoustic trauma causing hearing loss at the upper frequency range and around 4 000 Hz, corresponding to the regions of poorer *blood supply* (Ward et al, 1959, Bredberg 1968, Flottorp 1973)

Unfortunately there is very little knowledge about hearing in man below 125 Hz, because this is the lowest frequency in most audiometers The localization of 125 Hz in cochlea is at the transition *between the* second and third coil Hearing corresponding to the apical coil is therefore almost never the object of clinical examination The blood supply to this part is rather poor, being the last place of irrigation of A spiralis modioli (ramus tertius, RT) *According* to Bredberg (1968) degeneration of the organ of Corti at the apical turn is found rather frequently

Consequently a close correlation seems to exist between the area in cochlea with abundant blood supply and the frequency range for which hearing first develops and which during degeneration persists longest On the other hand, deterioration of hearing is found to start in the frequency range corresponding to regions with poor blood supply

## ACKNOWLEDGMENT

Mink kits were generously supplied by Assoc Prof Arne Helgebostad Research Station for Fur bearing Animals The Veterinary College of Norway Oslo and Assoc Prof Hans Rimeslåtten Department of Poultry and Fur Animal Science The Agricultural University of Norway Ås Assoc Prof Martin Davanger, M D and University Lecturer Tor Flage, Eye Department University Hospital Rikshospitalet Oslo kindly performed the ophthalmoscopy

## ZUSAMMENFASSUNG

Die Aufnahme des Gehorfunktion und das Öffnen der Augenlider wurden bei Katze Kaninchen Hund und Nerz untersucht Die Gehorteste wurden mit reinen Tonen und Larmbandern durchgefuhrt wobei man das Verhalten der verschiedenen Arten beobachtete Es wurde festgestellt dass das Gehor bei einem mittleren Lebensalter von 5 Tagen bei der Katze, von 7 Tagen beim Kaninchen 14 Tagen beim Hund und 29 Tagen beim Nerz auftrat Die Frequenzen auf die zuerst reagiert wurden und die nachfolgende Entwicklung des Gehors fur andere Frequenzen wurden angefuhrt und mit Daten in der Literatur fur funf andere Arten verglichen Die Augenlider offneten sich bei einem mittleren Alter von 7 Tagen bei der Katze von 10 Tagen beim Kaninchen 14 Tagen beim Hund und 33 Tagen beim Nerz Der mogliche Zusammenhang zwischen Blutzufuhr zu den verschiedenen Windungen der Schnecke (beim Menschen) und der ontogenetischen Entwicklung des Gehors und der Herabsetzung des Gehors im Alter und nach einem akustischen Trauma wurde erortert

## REFERENCES

Alford B R & Ruben R J 1963 Physiological behavioral and anatomical correlates of the development of hearing in the mouse *Ann Otol 72* 237

Andersen A C 1970 *The Beagle as an experimental dog* Iowa State University Press Ames Iowa

Anderson H & Wedenberg E 1965 A new method for hearing tests in the guinea pig *Acta Otolaryng* (Stockh) *60* 375

Anggård L 1965 An electrophysiological study of the development of cochlear functions in the rabbit *Acta Otolaryng* (Stockh) Suppl *203*

Asai K 1908 Die Blutgefasse im hautigen Labyrinthe des Hundes *Anat Hefte 36* 369

Axelsson A 1968 The vascular anatomy of the cochlea in the guinea pig and in man *Acta Otolaryng* (Stockh) Suppl *243*

von Bekesy G 1960 *Experiments in hearing* McGraw Hill New York

Boothroyd A & Cawkwell S 1970 Vibrotactile thresholds in pure tone audiometry *Acta Otolaryng* (Stockh) *69* 381

Bredberg G 1968 Cellular pattern and nerve supply of the human organ of Corti *Acta Otolaryng* (Stockh) Suppl *236*

Chaloupka Z Zahlava J, Myslivecek J & Hassmannova, J 1968 Development of functional abilities of the auditory system In *Ontogenesis of the brain* (ed L Jílek & S Trojan) p 387 Charles University Press Prague

Charachon R 1961 *Anatomie de l artere auditive interne chez l homme* These Lyon

Contino A 1907 Über Bau und Entwicklung des Lidrandes beim Menschen *Albrecht v Graefes Arch Ophthal 66* 505

Crowley D E & Hepp-Reymond M C 1966 Development of cochlear function in the ear of the infant rat *J Comp Physiol Psychol 62* 427

Djupesland G 1967 *Contractions of the tympanic muscles in man* Universitetsforlaget Oslo

Eichler O 1892 Anatomische Untersuchungen uber die Wege des Blutstromes im menschlichen Ohrlabyrinth *Abh Sachs Akad Wiss Math Phys Kl 18* 309

Erichsen S 1969 *Forsøksdyrbiologi En oversikt for dyreekspertimentell forskning* (*Biology of experimental animals A survey for experimental animal research*) Universitetsforlaget Oslo

Flottorp G 1973 Effects of noise upon the upper frequency limit of hearing *Acta Otolaryng* (Stockh) *75* 329

Flottorp G & Foss I 1970 The development of deafness in hereditary deaf white mink (Hedlund) (Hearing measurement and morphological examination of the inner ear in Hedlund and Standard mink) *Abstr X Int Congr Audiol* p 43 Karger, Basel

Foss I & Flottorp G 1970 Measurements of hearing and morphological examination of the inner ear in deaf white mink (Hedlund) and normal mink (Standard) *Proc 7th Int Congr Electr Micr 3* 761 Paris

Graham A B 1967 *Sensorineural hearing processes and disorders* Henry Ford Hospital International Symposium J & A Churchill London

Hall V E & Pierce G N Jr 1934 Litter size birth weight and growth to weaning in the cat *Anat Rec 60* 111

Hilding D A Sugiura A & Nakai Y 1967 Deaf white mink Electron microscopic study of the inner ear *Ann Otol 76* 647

Hunt W E & Goldring S 1951 Maturation of evoked response of the visual cortex in the post natal rabbit *Electroenceph Clin Neurophysiol 3* 465

Jasper H H Bridgman C S & Carmichael L 1937 An ontogenetic study of cerebral electrical potentials in the guinea pig *J Exp Psychol 21* 63

Johansson B 1972 Personal communication

Johansson B Wedenberg E & Westin B 1972 Continued tests of the tone response by human foetus 32 high risk cases Presented at *XI Int Congr Audiol* Budapest

Larsell O McCrady E Jr & Zimmermann A A 1935 Morphological and functional development of the membranous labyrinth in the opossum *J Comp Neurol 63* 95

Larsell O McCrady E Jr & Larsell J F 1944 Development of the organ of Corti in relation to the inception of hearing *Arch Otolaryng* (Chic) *40* 233

Lyngset A & Lyngset O 1970 Kullstørrelse hos hund (Litter size in the dog) *Nord Vet Med 22* 186

Marty R 1962 Développement post natal des réponses sensorielles du cortex cérébral chez le chat et le lapin Aspects physiologiques et histologiques *Arch Anat Micr Morph Exp 51* 129

McCrady E Jr 1938 *The embryology of the opossum* Wistar Institute Press Philadelphia

McCrady E Jr Wever E G & Bray C W 1937 The development of hearing in the opossum *J Exp Zool 75* 503

— 1940 A further investigation of the development of hearing in the opossum *J Comp Psychol 30* 17

Mikaelian D & Ruben R J 1965 Development of hearing in the normal CBA J mouse Correlation of physiological observations with behavioral responses and with cochlear anatomy *Acta Otolaryng* (Stockh) *59* 451

Nabeya D 1923 A study in the comparative anatomy of the blood vascular system of the internal ear in mammalia and in homo (Japanese) *Acta Sch Med Univ Kioto 6* 1

Parry H B 1953 Degenerations of the dog retina I Structure and development of the retina of the normal dog *Brit J Ophthal 37* 385

Pujol R Granier M R & Marty R 1967 Réponses électrocorticales à la stimulation tonale et développement de la cochlée *J Physiol* (Par) *59* 478

Pujol R & Marty R 1968 Structural and physiological relationships of the maturing auditory system In *Ontogenesis of the brain* (ed L Jílek & S Trojan) p 377 Charles University Press Prague

Rimeslåtten H 1973 The Agricultural University of Norway Personal communication

Rose G H 1968 The comparative ontogenesis of visually evoked responses in rat and cat In *Ontogenesis of the brain* (ed L Jílek & S Trojan) p 347 Charles University Press Prague

Rose J E Adrián H & Santibáñez G 1957 Electrical signs of maturation in the auditory system of the kitten *Acta Neurol Lat Amer 3* 133

Rose G H & Ellingson R J 1968 The comparative ontogenesis of visually evoked responses in rat cat and human infant *Electroenceph Clin Neurophysiol 24* 284

Scuderi R & del Bo M 1952 La vascolarizzazione del labirinto umano (The vascularization of the human labyrinth) *Acta Ital Otol 63* Suppl *11*

Siebenmann F 1894 *Die Blutgefässe im Labyrinthe des menschlichen Ohres* J F Bergmann Wiesbaden

Stevens S S Warshofsky F & the Editors of *Life* 1965 *Sound and hearing* Time Inc New York

Suguira A & Hilding D A 1970 Cochleo-saccular degeneration in Hedlund white mink *Acta Otolaryng* (Stockh) *69* 126

Udris A 1970 Minkvalparnas vikt vid födelsen (Weight of the mink kits at birth) *Vdra Pelsdjur 41* 151

Venge O 1953 Studies of the maternal influence on the growth in rabbits *Acta Agric Scand 3* 243

Volokhov A A & Shilyagina N N 1968 Correlations in the functional development of the cortical and subcortical divisions of the visual system in early ontogenesis of the rabbit In *Ontogenesis of the brain* (ed L Jílek & S Trojan) p 367 Charles University Press Prague

Wada T 1923 Anatomical and physiological studies on the growth of the inner ear of the albino rat *Amer Anat Mem 10* 1

Ward W D Glorig A & Sklar D L 1959 Relation between recovery from temporary threshold shift and duration of exposure *J Acoust Soc Amer 31* 600

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# SURGICAL TREATMENT OF DROOLING

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*Abstract* Eleven persons with brain damage and embarrassing drooling have been operated on by a retropositioning of the submandibular ducts combined with section and ligation of the sublingual ducts as a treatment against the drooling Results of the treatment have been analysed by objective measurements and subjective judgments of drooling Function studies of the submandibular glands have also been made pre and postoperatively Nine of the 11 patients show by objective measurement of drooling a postoperative improvement The subjective judgment often demonstrated diverging results by different nursing personnel within the same patient No signs of postoperative atrophy were seen in the function studies of the submandibular glands

Drooling is seen as a symptom in certain neurological diseases, e g Parkinson's disease, myasthenia gravis, cerebral palsy (CP) and is probably caused by hypersalivation and/or disturbances of the swallowing mechanism (Wilkie, 1967, Goode & Smith, 1970) Every year 200–300 children with CP are born in Sweden and about 10% of them will show an embarrassing drooling even after infancy (Enfors & Lundberg, 1968) This means that the contribution from this sector alone is 20–30 new cases a year While drooling is also seen in other neurological diseases there must be several hundred individuals in this country who need to be treated against drooling

Earlier studies on treatment of drooling have been few, covering a small number of patients and the estimation of the results has been made by subjective methods only There is without doubt a considerable disproportion between the magnitude of the problem and the resources that have been used to help these individuals

The therapy against drooling through the years has been anticholinergic drugs, mostly atropine The drawback to this therapy is that the administered dose often has to be so high that unwished side effects are seen, e g loss of visual accommodation, headache, dryness of the eyes and urinary retention (Goode & Smith, 1970) As a consequence various methods of surgery have been tried, e g. extirpation of the submandibular glands (Wilkie, 1967), retropositioning of salivary gland ducts (Wilkie, 1967, Laage Hellman, 1969) and sectioning of the efferent parasympathetic nerves to the parotid, submandibular and sublingual glands (Yoel & Markman, 1963, Goode & Smith, 1970) Combinations of the different methods are also described (Enfors & Lundberg, 1968, Wilkie, 1967)

The surgical method described in this paper is a retropositioning of the submandibular ducts combined with a ligation and division of the sublingual ducts The technique is the same as that described by Laage Hellman (1969) except for the division of the sublingual ducts

The purpose of the present investigation is

1 To estimate the results of surgical treatment of drooling by the help of the earlier described method for quantitative measurement of drooling (Ekedahl & Hallen, 1973)

2 To compare the author's quantitative

method with the subjective judgments made *by the nursing personnel The latter method* corresponds to the estimations of the treat ment results described in other articles (Yoel & Markman 1963 Wilkie 1967 Enfors & Lund berg 1968 Laage Hellman 1968 Goode & Smith 1970)

3 To ensure that a good postoperative re sult would not be caused by atrophy of the sub mandibular glands because the operative method used could result in kinking of the submandibular ducts with a possible secundary *glandular atrophy*

## MATERIAL AND METHODS

Eleven patients with brain damage mainly in the form of cereb al palsy and severe drooling were examined The age distribution was 7-46 years 5 female and 6 male The intellectual capacity varied from idiocy to normal and 9 of the patients lived in an institution for men *tally retarded Pre and postoperatively the* patients were examined with objective meas urements and subjective judgments of drool ing as well as function studies of the submandi bular glands

The patients were operated on under gen eral anesthesia Using a microscope an in cision was made around the papilla of the submandibular duct which was then prepared *from the surrounding tissues to the connection* of the sublingual duct In all the cases operated on one main duct from each sublingual gland was found After ligation and sectioning of this duct the submandibular duct was pre pared so that 2 3 cm were freely movable With the help of a pair of scissors a submucous *tunnel was made in the floor of the oral cavity* backwards to the palatal arches where an inci sion was made With a clamp the duct was brought through the tunnel and the papilla was fixed in the incision opening by a 4/0 atraumatic chromic gut suture The whole procedure was then repeated on the other side whereafter the mucosal *defect in the floor of* the mouth was sutured Antibiotics were not given as a routine

### *Objective measurement of drooling*

Quantitative measurements of drooling were performed preoperatively and 1 year post operatively 100 $\mu$Ci of the isotope $^{99m}$Tc was given intravenously to the drooling patients Saliva samples were taken every half hour 120-540 minutes after the isotope injection The drooled saliva was collected every half hour between the saliva samples in disposable bibs *(Molnlycke art 720500) The radioactivity in* the saliva samples was determined in a spectrometer type Picker Autowell with a 3 inch well crystal of sodium iodide while the bibs were examined in a Packard Model 3002 TRI-CARB scintillation spectrometer and an Armac Model 440 scintillation detector With the knowledge of radioactivity in the bibs and the saliva sam ples the amount of drooled saliva could be cal culated *The method is described in detail in* an earlier article by Ekedahl & Hallen (1973)

### *Subjective judgment of drooling*

Every patient was judged preoperatively and 1 year postoperatively by 3 persons in the nurs ing staff with good knowledge of and experi ence from the nursing of each patient The drooling was judged in three different situa tions at rest at mealtime and *in an engaged* situation e g at play In each situation it was *graded as insignificant* = 1 moderate = 2 rich = 3 or very rich = 4 units The mean values from these judgments by the 3 persons of the nursing personnel were calculated for each situation and compared pre and post operatively A difference of more than 1 unit was classified as an improvement or impair *ment From these differences* the patients could be judged as free of drooling improved unimproved or impaired Independent of this differentiated grading 3 persons of the nurs ing staff on another occasion postoperatively made an integrated judgment as to whether the patient was free of drooling improved un *improved or impaired*

Table I *Results from pre- and postoperative objective measurements of drooled saliva and comparison with the results of the integrated subjective judgment*

| Patient | Age | Sex | Objective measurement: Preop [a] | Postop [a] | Difference[b] | % Improvement | Integrated subjective judgment |
|---|---|---|---|---|---|---|---|
| K A | 10 | ♀ | 5 25 | 0 80 | 4 45* | 84 | Improved |
| C P | 11 | ♀ | 2 11 | 0 18 | 1 93* | 91 | Improved |
| B J | 18 | ♀ | 9 90 | 1 41 | 8 49* | 85 | Unimproved |
| L U | 18 | ♀ | 9 09 | 2 33 | 6 69* | 73 | Improved |
| B O | 19 | ♀ | 5 50 | 2 39 | 3 11* | 56 | Unimproved |
| A L | 46 | ♀ | 3 92 | 0 28 | 3 64* | 92 | Improved |
| M S | 7 | ♂ | 3 82 | 1 32 | 2 50* | 65 | Unimproved |
| P J | 12 | ♂ | 3 05 | 2 95 | 0 10 | 3 | Improved |
| J P | 21 | ♂ | 4 30 | 0 76 | 3 54* | 82 | Impaired |
| L S | 21 | ♂ | 3 86 | 3 22 | 0 64 | 16 | Unimproved |
| B K | 26 | ♂ | 8 19 | 3 77 | 4 42* | 53 | Improved |
| Mean | | | 5 35 | 1 76 | | 64 | |

* Significance at a 5% level
[a] Each value expressed in g/30 min is the mean of 42 measurements
[b] Difference between pre- and postoperative values

*Control of function of submandibular glands*

In order to exclude kinking of the retropositioned submandibular ducts, resulting in glandular atrophy, function of the glands has been controlled by scintigraphy The patients got 0 25–0 50 mg atropine subcutaneously just before the administration of 200 $\mu$Ci $^{99m}$Tc in order to reduce the uptake of the isotope in the small acinary glands of the oral mucosa (Emmelin, 1967, Enfors et al, 1969) Twenty minutes after the technetium injection a scintigram was made with a Picker Magnascanner III with a 19 hole coarse focusing collimator The scintigram was taken in the frontal position Immediately after the scintigram had been taken, a quantitative measurement expressed in counts was made for 1 min over the submandibular glands with the patient in the same position (McG Harden et al, 1967) The same procedure was made 6–12 months after the operation and was compared with the preoperative measurement

## RESULTS

*Objective measurement of drooling*

The results of the pre- and postoperative measurements are shown in Table I Because of the earlier described variations in amounts of drooled saliva (Ekedahl & Hallén, 1973) 42 measurements preoperatively and an equal number postoperatively have been required to get an acceptable precision in the estimate of the mean The comparison of the values is based on a $t$ test with paired observations of the means from all the measurements on each patient The statistical significance is tested at a 5% level The results from the $t$ test for the whole group shows $t = 4\ 88$ for 10 degrees of freedom, which means a difference between the pre and postoperative results that is statistically significant Examining each patient separately in the same way, differences pre- and postoperatively in 9 of 11 patients are statistically significant and in these 9 patients the percentage improvement varies from 53 to 91%

*Subjective judgment of drooling*

The results from the differentiated and integrated judgments are shown in Table II The differentiated judgments made by 3 persons of the nursing personnel in the 3 different situations show an improvement on all counts in only 2 of the 11 patients There is a uniformity in the estimations in only 2 more cases, indicating no postoperative improvement The

Table II *Results from subjective estimation of drooling pre- and postoperatively*

| Patient | Differentiated judgment | | | Integrated judgment |
|---|---|---|---|---|
| | At rest | At mealtime | Engaged situation | |
| K A | Unimproved | Improved | Improved | Improved |
| C P | Improved | Improved | Improved | Improved |
| B J | Improved | Worsened | Improved | Unimproved |
| L U | Unimproved | Improved | Unimproved | Improved |
| B O | Unimproved | Unimproved | Unimproved | Unimproved |
| A L | Unimproved | Improved | Improved | Improved |
| M S | Unimproved | Unimproved | Unimproved | Unimproved |
| P J | Unimproved | Unimproved | Improved | Improved |
| J P | Improved | Worsened | Improved | Worsened |
| L S | Unimproved | Worsened | Improved | Unimproved |
| B K | Improved | Improved | Improved | Improved |

remaining 7 patients generally show great variations in the differentiated judgment Four and 5 patients respectively are improved at rest" and at mealtime" while 8 are considered to be improved in engaged situations" The integrated judgments made by 3 persons of the nursing staff with the purpose of getting a conclusive evaluation of the postoperative results show an improvement in 6 of the 11 cases The differentiated and integrated judgments are in agreement only in those 4 patients who showed a uniformity in the differentiated judgment The integrated subjective judgment is compared with the results from the quantitative measurement in Table I and there is a lack of coordination in not less than 5 of the patients

Table III *Isotope uptake ($^{99m}Tc$) in the submandibular glands pre- and postoperatively (expressed in cpm)*

| Patient | Preoperatively | | Postoperatively | |
|---|---|---|---|---|
| | Right | Left | Right | Left |
| K A | 8 951 | 9 138 | 4 894 | 5 901 |
| C P | 11 265 | 9 841 | 8 135 | 7 913 |
| B J | 3 514 | 3 076 | 3 951 | 3 432 |
| L U | 4 526 | 5 320 | 3 349 | 5 471 |
| B O | 5 499 | 4 668 | 4 839 | 5 201 |
| A L | 2 159 | 2 337 | 5 246 | 5 508 |
| M S | 6 732 | 6 009 | 9 163 | 9 503 |
| P J | 9 596 | 10 012 | 5 303 | 8 841 |
| J P | 3 445 | 3 594 | 5 221 | 5 722 |
| L S | 3 304 | 3 245 | 6 869 | 6 855 |
| B K | 3 710 | 4 174 | 4 282 | 4 694 |

*Function of the submandibular glands*

The results from the isotope uptake measurements of $^{99m}Tc$ in the submandibular glands are seen in Table III The time interval between the operation and the postoperative scintigraphy is 6–11 months (mean 10 months) In 10 of the 11 cases no reduction is seen in the postoperative values compared with the preoperative In case K A a postoperative reduction is seen in both glands which probably is the result of different functional states. This seems still more probable as many of the patients show higher postoperative count rates than the preoperative ones The background activity has also been tested in some of the patients directly after the scan had been taken and the figures did not exceed 1 100 cpm Thus the results speak in favour of a good function of the submandibular glands in the patient K A

## DISCUSSION

The probability of a combination of several etiological factors in cases of drooling makes the choice of therapy difficult Irradiation of the salivary glands should be avoided on these indications The medical therapy has been transcribed because of the disadvantage earlier described in this paper and furthermore many of the 11 patients have already been treated with anticholinergics mostly without success The aim of the author has been to use a surgical method which is minimally destructive As

Wilkie (1967) showed a disturbance in the saliva transport from the frontal parts of the mouth to the pharynx in children with cerebral palsy and drooling, it seemed wise to try to overcome this problem by letting the saliva from the submandibular ducts fall directly into the pharynx Another reason for using the actual surgical method is that most of the drooling occurs during a state of resting secretion', which in 60–70% comes from the submandibular and sublingual glands (Schneyer & Levin, 1955, Rauch, 1959) For the above-named reasons the patients have been operated on with a bilateral retropositioning of the submandibular ducts combined with a ligation and *sectioning of the main ducts to the sublingual* glands

No complications were seen during the operations In 1 case a postoperative haematoma was seen in the floor of the mouth causing slight, transitory respiratory problems Fluids or mashed food could generally be accepted already during the first postoperative day and the patients could be sent to their homes or nursing homes 1–2 days after the operation In no case was a distressing swelling of the submandibular or sublingual glands to be seen

The study shows that 9 of the 11 patients are improved by the operation, as measured by the objective method The one of the unimproved patients (L S) has a poor intellectual faculty, a spastic tetraplegia and marked difficulties in swallowing as shown in an earlier article (Ekedahl et al, 1973) The patient was described by the personnel postoperatively as unimproved

The second patient (P J) has a spastic diplegia, is mentally very poor but has no serious disturbance of the swallowing mechanism This patient almost always sits bent forward with his mouth open, which probably explains the poor result of the operation Both of the unimproved patients were treated with benzodiazepines which, according to Hagberg (1968) and Eeg Olofsson (1973), could result in hyper salivation

In the subjective differentiated judgment the study often shows divergent estimations of drooled saliva by different nursing personnel within the same patient The integrated judgement also shows great differences in the estimated treatment results, as reported by various members of the nursing staff Apart from these facts only a weak correlation is seen between the differentiated and integrated judgments The results indicate a marked uncertainty concerning the subjective measurement of drooling which thus could not possibly constitute a basis for comparison of the results from different kinds of treatment

It has been questioned if the retropositioning of a salivary gland duct could result in kinking of the ducts and if so, will the result be a glandular atrophy If the kinking results in total obliteration of the salivary gland duct, a glandular atrophy would be expected This opinion is supported by the fact that ligation of salivary gland ducts in rabbits and mice has resulted in histologically demonstrable glandular atrophy (Wallenborn et al, 1968, Junqueira, 1951, Standish & Shafer, 1957, Tamarin, 1967) After ligation of the duct of Stensen in a patient, Baron & Ober (1962) found atrophy of both the tubular and acinar cells in the treated gland

The uptake of $^{99m}$Tc is presumed to be reduced in an atrophied gland as in chronic sialoadenitis and Sjogren's syndrome with known salivary gland atrophy, where a reduced glandular uptake of $^{99m}$Tc has been shown (Fridrich & Wey, 1968, Grove & di Chiro, 1968, Schall et al, 1971) In the present study, there was, however, no reduced uptake in the submandibular glands 6–11 months after the operation, which must support a conclusion that the operative method used with retropositioning of the submandibular ducts has not resulted in kinking of the ducts, thus causing an atrophy of the glands

## ZUSAMMENFASSUNG

Elf Personen mit Gehirnschaden und belastigendem Geifer sind durch eine Retroposition der Ausfuhrungsgange der Submandibulardrusen operiert worden kombiniert mit einer Ligatur und Abschneidung der Ausfuhrungsgange der Sublingualdrusen als Behandlung gegen den Geifer Die Resultate der Behandlung sind im Zusammenhang mit pra und postoperativen Untersuchungen einschliesslich objektiver Messung bzw subjektiven Schatzungen des Geifers und der Funktion der Submandibulardrusen analysiert worden Neun der elf Patienten weisen bei objektiver Messung des Geifers eine postoperative Verbesserung auf Die subjektiven Schatzungen von verschiedenem Behandlungspersonal bei demselben Patienten ergaben oft divergierende Resultate Keine Zeichen von postoperativer Atrophie konnten in den funktionellen Untersuchungen der Submandibulardrusen beobachtet werden


## REFERENCES


Baron H C & Ober W B 1962 Parotid gland atrophy Observations after ligation of Stensen s duct *Arch Surg* (Chic) *85* 1042

Eeg Olofsson O 1973 Experiences with Rivotril in treatment of epilepsy—particularly minor motor epilepsy in mentally retarded children *Acta Neurol Scand 1973* In press

Ekedahl C & Hallen O 1973 Quantitative measurement of drooling *Acta Otolaryng* (Stockh) *75* 464

Ekedahl C Månsson I & Sandberg N 1973 Swallowing dysfunction in the brain-damaged with drooling *Acta Otolaryng* (Stockh) In press

Emmelin N 1967 Nervous control of salivary glands In *Handbook of physiology* II Alimentary Canal

nfors B & Lundberg Å 1968 Behandling av hypersalivation hos cp-barn *Lakartidn 65* 4416

Enfors B Lind M & Soderborg B 1969 Salivary gland scanning with ⁹⁹ᵐtechnetium *Acta Otolaryng* (Stockh) *67* 650

Fridrich R & Wey W 1968 Beitrag zur Szintigraphie der Speicheldrusen *Schw Med Wochenschrift 98* 335

Goode R L & Smith R A 1970 The surgical management of sialorrhea *Laryngoscope 80* 1078

Grove Jr A S & Di Chiro G 1968 Salivary gland scanning with technetium ⁹⁹ᵐ pertechnetate *Amer J Roentgenol 102* 109

Hagberg B 1968 The Chlordiazepoxide Hcl (Librium) Analogue nitrazepam (Mogadon) in the treatment of epilepsy in children *Develop Med Child Neurol 10* 302

Harden R McG Hilditch T E Kennedy I Mason D K Papadopoulos S & Alexander W D 1967 Uptake and scanning of the salivary gland in man using pertechnetate ⁹⁹ᵐTc *Clin Sci 32* 49

Junqueira L C U 1951 Cytological cytochemical and biochemical observations on secreting and resting salivary glands *Exp Cell Res 2* 327

Laage Hellman J E 1969 Retroposition av glandula submandibularis utforsgång som behandling vid dragling *Nord Med 82* 1522

Rausch S 1959 *Die Speicheldrusen des Menschen* G Thieme Verlag Stuttgart

Schall G L Anderson L G Buchignani J S & Wolf R O 1971 Investigation of major salivary duct obstruction by sequential salivary scintigraphy *Amer J Roentgenol 113* 655

Schneyer L H & Levin L K 1955 Rate of secretion by individual salivary gland pairs of man under conditions of reduced exogenous stimulation *J Appl Physiol 7* 508

Standish S M & Shafer W G 1957 Serial histologic effects of rat submaxillary and sublingual salivary gland duct and blood vessel ligation *J Dent Res 36* 866

Tamarin A 1967 Secretory cell alterations associated with submaxillary gland duct ligation In *Secretory mechanisms of salivary glands* (ed Schneyer and Schneyer) Academic Press New York and London

Wallenborn W McK Hsu Y T & Olinger B R 1968 The experimental production of parotid gland atrophy *Laryngoscope 78* 1314

Wilkie T F 1967 The problem of drooling in cerebral palsy A surgical approach *Canad J Surg 10* 60

Yoel J & Markman I 1963 The surgical treatment of sialorrhea *J Internat Coll Surg 39* 261



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# IDIOPATHIC HEMIFACIAL SPASM

## *New Method of Surgical Treatment*

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*Abstract* A new surgical technique is presented for the treatment of the idiopathic hemifacial spasm It consists in exposing the nerve in the third portion of the Fallopian canal performing a longitudinal neurotomy and inserting a silicon rubber sheet through it to avoid nerve regeneration Comments are made concerning the etiology diagnosis and surgery of this disease Three cases are presented along with the results obtained

In the idiopathic hemifacial spasm one side of the face is agitated by paroxysmal clonic involuntary and episodic muscular contractions usually very noticeable in the lower eyelid and labial commissure This causes an unpleasant grimace that is aggravated under emotional tension making the patient feel miserable when being inquisitively watched by third persons

The affection must be clinically differentiated from the post paralytic hemifacial spasm (frequent sequelae of severe Bell s facial palsy not opportunely decompressed) and from hemifacial spasm symptomatic of a compressive lesion of the facial *nerve usually tumorous and located* in the cerebello pontine angle or temporal bone

Since it does not respond to medical therapy many surgical methods have been described for its treatment but none has been generally accepted In this article we will discuss the etiology of the disease its diagnosis and the different operative techniques that have been developed We will further propose a new technique that we hope will be useful in controlling this antiesthetic affection

The etiology of idiopathic hemifacial spasm is unknown There are several relevant theories

*Otologic thesis*

Many authors (Woltman et al 1951 Williams et al 1952 Proud 1953 Pulec 1972) believe the causative lesion is an edema of the facial nerve or a fibrous constriction of its sheath somewhere along its course through the Fallopian canal

*Neurosurgical thesis*

The lesion could be caused by continuous and prolonged slight pressure upon the seventh nerve by a vascular structure in the posterior cranial fossa (Gardner & Sava 1962 Janetta 1972)

*Neurological thesis*

The pathological condition could be located in the motor nucleus of the facial nerve in the brain stem (Wartenberg 1952 Crue et al 1968 Liebalt & Henn 1970)

Authors report dissimilar findings in defending *these various theories each submitting positive* proof in support of his ideas This suggests that idiopathic hemifacial spasm does not have a unique cause but that in different cases it may have different etiologies Clinically it is very difficult if not impossible to determine in a patient with that disease whether the lesion is located in the nucleus in the cerebello-pontine angle or in the Fallopian canal This circumstance must be taken into account when selecting a surgical technique

Physiopathology of this disease is better known A series of involuntary anomalous and

frequent, motor impulses travel through the facial nerve They may be electromyographically registered in the facial muscles and have well known characteristics (Isch & Jesel, 1967)

Supporters of the nuclear thesis believe these impulses originate in the ganglionic cells of the facial nerve in the brain stem, disturbances in the excitability threshold of those cells have been reported (Liebalt & Henn, 1970)

Those who accept the peripheral etiologic hypothesis postulate the existence of an irritating focus in any segment of the affected facial nerve This area behaves as an artificial synapse transforming the presynaptic orthodromic impulse into a series of postsynaptic impulses, reflecting the antidromic impulses in the fashion of a pseudoreflex, and "short-circuiting" all nerve impulses reaching the area to the adjacent nervous fibers (Isch & Jessel, 1967)

The operative techniques that have been developed can be classified in two groups One designed to act on the disease etiology, and the other, on its physiopathology

Those who favor the first technique, the supporters of the otologic thesis, perform a decompression of the facial nerve incising its perineurium

Initially, only the mastoid portion of the facial canal used to be decompressed (Woltman et al, 1951, Proud, 1953), later, as the otoneurosurgical techniques developed, the entire facial nerve was decompressed from the internal auditory canal to the stylomastoid foramen (Pulec, 1972) The majority of these authors report finding abnormalities such as edema, fibrous constriction of the perineurium, or diffuse thickening of it However, Cawthorne (1965), in thirteen cases, found no alternations of that nature Apparently, the results of this procedure depend upon the surgical traumatism on the nerve, the best results being obtained when the postoperative facial paralysis is more severe and long lasting

Supporters of the neurosurgical thesis manipulate the nerve in the posterior cranial fossa by the suboccipital approach In a remarkably high number of cases they have reported compression of the nerve by a vascular structure cirsoid aneurysm of the basilar artery, arterio venous malformation, loop of the anterior-inferior cerebellar artery (Laine & Nayrac, 1948, Gardner & Sava, 1962, Janetta, 1972) They manipulate the nerve in the cerebello-pontine angle, separating it from the vessel that may be eventually compressing it In using this technique it is possible to injure other nervous structures especially the eighth nerve, sometimes causing irreversible alterations Furthermore, it is difficult to see how it is possible to separate permanently a vascular structure of the cerebello pontine angle from the seventh nerve as it manipulation is delicate, and could cause th patient's death

The otologists' findings, as well as the neuro surgeons', are strictly subjective, being mos remarkable for the great contradictions existing between them

As it is impossible in these cases to establish the etiology preoperatively, other techniques tend to direct the treatment towards the physio pathology of the disease, i e to diminish the number of axons that transmit motor impulses which are abnormal, involuntary, frequent and of unknown origin

One of the oldest and most commonly used methods was alcohol injection of the nerve where it leaves the stylomastoid foramen (Harris & Dickson, 1932) This procedure has been recently modified by Wakasugi (1972) who traumatizes the nerve by inserting a needle in the facial trunk at the same point These methods produce a transitory facial paralysis and there fore, absence of the spasm during the time the same lasts But they lead inevitably to recurrence since they permit nervous regeneration, and what is worse, aggravate the spasm by creating a new irritating focus New artificial synapses are caused and also misdirection of the regenerated fibers

Another group of authors has developed techniques to partially and definitively decrease the number of axons, thus preventing its re generation Some surgeons approach the nerve in the parotid region, beyond its bifurcation,

performing longitudinal hemisections in the various branches Afterwards, they fold the sectioned fragment centrally, tying it to its trunk of origin (Miehlke, 1961, Diamant et al, 1967) Similar procedures have been devised for blepharospasm, except that these make a selective, definitive neurotomy instead (Reynolds et al, 1967, Harvey, 1971, McCabe & Boles, 1972) These techniques leave unsightly scars, and in case of recurrence, make it very difficult to reoperate by the same approach, since the cicatrization hinders the dissection of these thin branches (Diamant et al, 1967)

Analogous techniques have been described approaching the nerve in the mastoid process and performing longitudinal partial neurotomies The sectioned portion is folded centrally (Scoville, 1965), or buried in openings made, utilizing a burr (Lewis, 1965), in the mastoid bone in order to prevent regeneration

In this paper we present a new, simple and practical method that we have devised along these lines

## MATERIAL AND METHODS

### *Diagnosis*

Clinical characteristics of this disease have been described at length (Ehni & Woltman, 1945) We will only emphasize the parameters we apply to classify a spasm as idiopathic

(*a*) Absence of other otoneurologic symptomatology (excluding tinnitus and/or fugacious vertigo, since these may be present in any type of hemifacial spasm They are caused by contractions of the stapedius muscle, simultaneous with the spasm)

(*b*) Normal temporal bones X-ray study

(*c*) Normal posterior fossa X ray study (should include at least myeloencephalography)

(*d*) Normal audiologic and electronystagmographic study

(*e*) Absence of history of homolateral peripheral facial paralysis

The need to make all these tests is obvious Any hemifacial spasm may be symptomatic of an

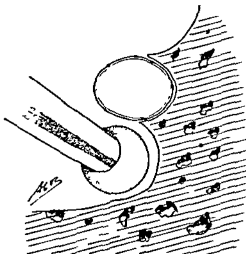


*Fig 1* Dissection of the facial nerve utilizing the burr Exposing the nerve 270°, lateral and posterior surfaces and slightly more than 50% of its medial surface

expanding lesion in the cerebello-pontine angle or of a tumor in the temporal bone

### *Technique*

To gain access to the third portion of the facial nerve, we use the transmastoid approach following the usual procedure of decompressing this portion of the nerve (Lewis, 1957) This technique is so well known that we will not describe it in detail

Mastoidectomy should be ample, skeletonizing the lateral sinus and thoroughly exposing the fossa incundis and the digastric ridge, thus providing more room and allowing better maneuvering at the facial nerve level

The seventh nerve is then skeletonized by using a cutting burr initially, and later, removing the last osseous layers with a diamond burr Thus, only a fine membrane is left covering the nerve and which can be later removed with a small hook Continuous suction irrigation should always be used Tympanotomy at the facial recess should be performed between the chorda tympani and the Fallopian canal in order to fully expose the third portion

The removal of bone around the facial nerve is performed in an extension of approximately 270° exposing very well the lateral, posterior, and

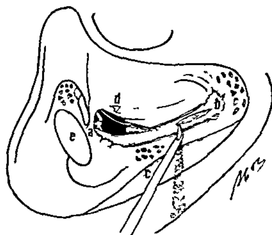



*Fig 2* Incision of the perineurium (*a*) Fossa incudis (*b*) digastric ridge, (*c*) lateral sinus, (*d*) posterior tympanotomy, (*e*) horizontal semicircular canal

medial surfaces of the nerve trunk The latter portion should be exposed by over 50% to permit performing the neurotomy (Fig 1)

Once the nerve is exposed, the perineurium is incised all along the third portion (Fig 2) Later, using a thin and very sharp knife, the neurotomy is performed dividing the nerve in half along its major axis (Fig 3) The extension of the incision is directly proportional to the spasm's severity After concluding the neurotomy, a very thin sheet of silicon rubber, of 0 12 mm gauge, is inserted in its sinus, slipping it gently between the two nerve trunk halves and folding its lower end backwards to prevent it from slipping out of the neurotomy (Figs 4 and 5) The length of this sheet depends upon

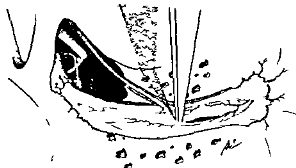


*Fig 3* Longitudinal medial incision of the facial nerve along its major axis

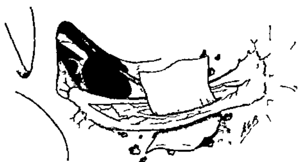


*Fig 4* Silicon rubber sheet inserted in the neurotomy

the size of the neurotomy and its width is of 1 cm

### *Clinical cases*

We have previously reported the methods used in exploring the facial nerve (Celis-Blaubach 1968)

### *Case 1*

A 62 year-old woman developed a right hemi facial spasm 1 year before examination No other symptoms were present Physical examina tion of the nose, throat, and ears was entirely normal, as was a neurological examination The

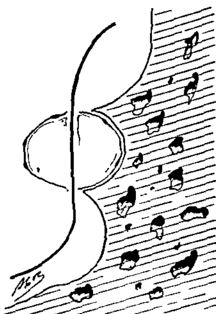


*Fig 5* Longitudinal section of the nerve, showing the position of the silicon rubber sheet between the two halves of the nerve

tear test showed 50 mm of saturation in 5 minutes on the right side, and 50 mm in 8 minutes on the left The stapedial reflex was absent on the right side Electric taste tests revealed a threshold of 38 $\mu$A on each side, and chemical gustometry was within normal limits Excitability test showed a threshhold of 4 mA on each side Audiometry, tympanometry and electronystagmography were normal Plain X-rays of the temporal bone and positive contrast myelography of the posterior fossa, showed no abnormality On October 15th, 1970, surgery, following the procedure here reported, was performed No edema or alterations of the facial nerve sheath were found A neurotomy of 5 mm was done Facial paresis appeared immediately after surgery After 1 month, facial motility was almost normal Six months following surgery slight fibrillar muscular contractions appeared on the right lower lid Two years after the operation these were still present, but had not worsened

*Case 2*

A 58-year-old woman, had left hemifacial spasm of progressively increasing severity for 6 years No other complaints were presented Physical examination of the nose, throat and ears was within normal limits Neurological examination was also normal Schimmer's tests revealed 50 mm of saturation in 10 minutes on the right side and 50 mm, but in 90 seconds, on the left Stapedial reflex was present on both sides Electrogustometry showed a threshold of 50 $\mu$A on each side and the gustogram was normal Excitability test revealed a threshold of 5 mA on the right side, and of 6 mA on the left Audiometry, tympanometry and electronystag mography showed no abnormalities Plain X ray and positive contrast roentgenography of the posterior fossa, were normal On April 1st, 1971 surgery following the technique here described was performed Facial nerve showed no alteration Since the spasm was extremely severe, an extensive neurotomy (all along the mastoid portion of the facial nerve) was performed Severe facial paralysis appeared immediately after surgery Two months later, first voluntary facial movements began to appear, and 7 months later, only moderate facial paresis persisted, visible when making extreme grimaces Seventeen months postoperatively there was not the slightest sign of recurrence

*Case 3*

A 22-year old woman complained of left hemifacial spasm of 20 months' duration Since the onset this symptom was accompanied by left tinnitus and occasional vertigo, simultaneous with the spasmodic crisis Physical examination of the nose, throat and ears, were within normal limits Neurologic examination revealed a moderate decrease of the cutaneous sensibility in the left fifth nerve area Nevertheless, left corneal reflex was normal The tear tests were symmetric The stapedial reflex was absent on the left side Electric taste tests revealed a threshold of 64 $\mu$A on the right side and 8 $\mu$A on the left Gustogram was normal Audiometry, tympanometry and electronystagmography were within normal limits Plain X-rays of the temporal bones and the cervical column, and a left vertebral and carotid arteriography, were normal Pneumoencephalography showed a slight filling defect in the left cerebello-pontine cistern, but myeloencephalography and brain scanning were normal On June 11th, 1970, a left facial nerve decompression, utilizing the mastoid approach, was performed Perineurium was incised and several small vertical neurotomies were made in the facial nerve (Portmann, M , 1968) Slight facial paresis appeared immediately after surgery and the spasm disappeared Only small fasciculations on the left lower lid were noticeable One year after surgery the hemifacial spasm recurred being even more severe On August 3rd, 1972 a second operation, following the procedure we described in this paper, was carried out A 1 cm neurotomy was made The spasm disappeared and the postoperative facial palsy lasted 2 months Six months after surgery she was feeling well, without evidence of spasm



Fig 4 With a pair of scissors or hemostats the instrument can easily be angled to a desired shape

angle on a cutting instrument would be helpful By using an injection needle on this handle and a pair of scissors, or hemostats or forceps, a sharp instrument of most desired angles and shapes can easily be produced

## ZUSAMMENFASSUNG

Der Sauger im HNO Dienst ist zum Saugen sowie als ein mechanisches Instrument zu verwenden Dies stellt besondere Anforderungen an die Starke des Saugers Heute konnen nur Metallsauger fur mehrmaligen Gebrauch diesen Anforderungen entsprechen Trotz sorgfaltiger Reinigung blockiert der Sauger im allgemeinen nach wiederholter Verwendung Um den Anforderungen zu entsprechen und die Nachteile auszuschalten ist ein Metallsauger fur einmaligen Gebrauch entwickelt worden

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# ULTRASTRUCTURE OF THE COCHLEAR BLOOD VESSELS

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*Abstract* The ultrastructure of the cochlear blood vessels from the artery to the vein has been investigated Some major differences between the small vessels of the modiolus and those of the membranous labyrinth are noted Morphological evidence suggests that the major control of blood flow to the organ of Corti is accomplished at the modiolus Fenestrated capillaries are seen among the modiolar cells which are unusually rich in cell organelles These vessels are probably the major source of modiolar fluid and may be engaged in reduction of blood pressure and pulsatile movement of the blood vessels Intracellular filaments in bundle form are common in the endothelial cells of the artery, arterioles and the arteriovenous arcades Their presence is associated with vessels in which blood flow is very fast Capillaries of the stria vascularis come directly in contact with marginal, intermediate and basal cells The venules and inferior cochlear vein are surrounded by widely spaced pericytes or primitive smooth muscle cells and scant fibrocytes Nerve fibers are lacking adjacent to the vessels of the membranous labyrinth with the exception of the tympanic lip of the limbus spiralis

The ultrastructure of blood vessels in various organs has been described in great detail and reviewed by numerous investigators (Bennett et al, 1959, Farquhar, 1961, Palade, 1961, Fawcett, 1963, Luft, 1965 Majno, 1965, Rhodin, 1968) In the inner ear some fine structural studies of blood vessels have been reported by Smith (1957), Hinojosa & Rodriguez Echandia (1966), Hawkins (1967) Iurato (1967), Kimura & Schuknecht (1970), and Duvall et al (1971) More recently, a comparative study of the labyrinthine vessels of different species has been reported by Wersall et al (1973) The present study is intended to provide a full description of the entire vascular system of the cochlea from the arterial to the venous sides indicating morphological differences along the course of the vessels

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## MATERIALS AND METHODS

Thirty-three guinea pigs weighing 300 to 400 grams were used for this study The specimens were fixed in 1 % phosphate-buffered osmium and others were fixed initially with Karnovsky's fixative (1965) and post-fixed with osmium The fixatives were perfused through the opened oval and round windows For the modiolar vessels, the fixatives were pumped in from the internal auditory canal after the apical part of the cochlea was opened The blood vessels encased in bone were prepared both undecalcified or decalcified in 10% EDTA The specimens were embedded in Epon, cut with an LKB ultrotome, double stained with uranyl acetate and lead citrate, and examined with a Siemens Elmiskop I at magnifications from 1 000 to 40 000 × The diameter of the vessels is recorded from mid point to mid point of the endothelial cells

## FINDINGS

The cochlear artery is a branch of the inferior cerebellar artery and ascends toward




*Fig 1* Main cochlear artery showing endothelial cells (*E*) elastic layer (*EL*) smooth muscle cells (*S*), and adventitia composed of fibrocytes (*F*) and collagen fibers (*C*) Note unmyelinated nerve fibers (*arrow*) × 6 000

the apex, spiralling around the cochlear nerve trunk The artery is about 83 μ at the base of the modiolus and becomes gradually smaller as tributaries are sent out toward the membranous labyrinth The arterial wall is composed of endothelial cells, an elastic layer, one to three layers of smooth muscle cells and adventitia (Fig 1) The endothelial cell is tall (2 to 4 μ) and contains an enormous number of filaments (67 Å in diameter) Mitochondria and rough endoplasmic reticula are few, but many RNA particles are recognized A few light and dense oval masses and multivesiculated bodies are also noted Pinocytotic vesicles are small in number and are located mostly at the basal surface At the luminal surface marginal folds and a few microvilli are observed The profile of the cell junction is often straight, vertical to the cell surface, but other forms such as straight slant, C, S, or Y types of interlockings are also seen The tight junction is often long or intermittent Another type of junction is the simple abutment of plasma membranes with condensation of adjacent cytoplasms

The basement membrane of the cochlear artery is continuous except in areas where the smooth muscle cells establish a tight junction with the endothelial cells, and where intercellular fibrils abut endothelial cells It extends outward into the elastic layer in a honeycomb arrangement The elastic layer is about 1 μ in thickness and is interspersed with fibrils or tubules (127 Å) which follow the extension of the basement membrane Collagen fibers (427 Å) are sparse on the endothelial side but numerous at the periphery of the smooth muscle layer Unmyelinated nerve fibers are common and contain many vesicles but vesicles with a core are rarely seen The

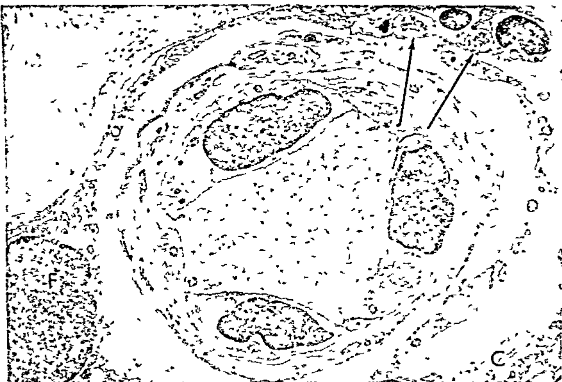



*Fig 2* An arteriole (8 8 $\mu$ diameter) of the modiolus. Smooth muscle cells completely surround the endothelial cells Outside the vessels are patches of collagen fibers (*C*), veils of fibrocytes (*F*) and a few unmyelinated nerve fibers (*arrow*) surrounded by a Schwann cell ×7 000

nerve fibers come close to the smooth muscle, but are never seen attached to it Outside the collagenous layer are fibrocytes which form a series of thin cell processes (veils), in as many as seven layers, following the curvature of the vessel Melanocytes are frequently seen around the arteries Their cell processes appear to touch the basement membrane of the smooth muscle cells Presumably, they are involved in phagocytosis

Before leaving the modiolus for the membranous labyrinth, the smaller cochlear arteries and arterioles twist and loop within the stroma of connective tissue cells These vessels retain distinct smooth muscle cells down to the size of 5 $\mu$ (Fig 2) In most cases the vessels are obviously contracted as evidenced by a wavy basal surface The most prominent feature of the arteriolar endothelial cell is the presence of numerous filament bundles Other cell organelles are similar to those of the larger arteries Pinocytotic vesicles are located more on the basal surface The elastic layer becomes thinner but is still recognizable down to the size of 7 $\mu$ The smooth muscle layer is thin and dense foci within the cytoplasm become exceedingly difficult to visualize The smooth muscle makes contact with the endothelial cell by extending small cell processes to the flat cell surface or to the invagination, also, the endothelial cells send processes to the smooth muscle cells The adventitia is similar to that of larger arteries but the number of fibrocytes and collagen fibers is greatly decreased. Unmyelinated nerve fibers are found in the area (distance about 0 2 $\mu$) adjacent to the arterioles up to 5 $\mu$ in size, they are partially or completely surrounded by Schwann cells (Fig 2)

An unexpected finding is fenestrated vessels in the modiolar connective tissue (Figs 3 A, 4) Their diameter is about 5 3 $\mu$, and




*Fig. 3.* (A) Coiled vessels of the modiolus surrounded by a series of the attenuated cytoplasm of modiolar cells (*M*). The vessel at the upper left corner is a fenestrated type. ×5 000. (B) Unmyelinated nerve fibers (*arrow*) are surrounded by concentrically arranged cell processes of modiolar cells (*M*) at some distance away from the blood vessels ×8 000.

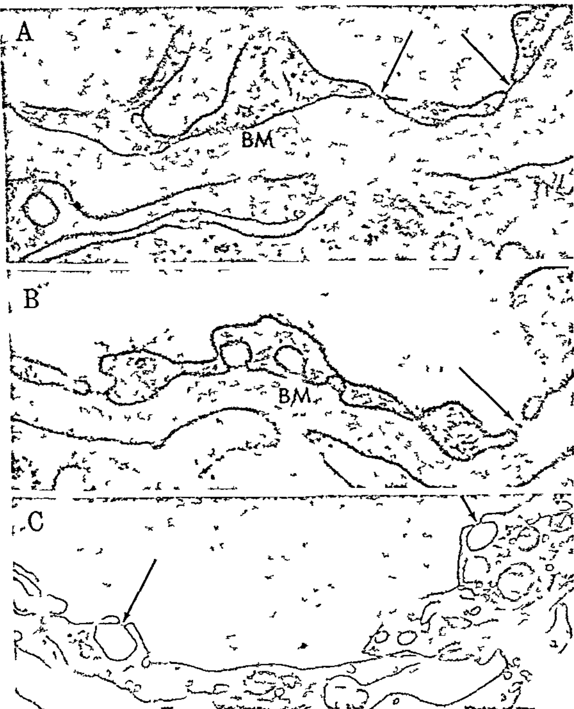



*Fig 4* (A) A fenestrated port on (*arrows*) of the mod olar cap llary endothel um The fenestra measures about 550 Å across. Basement membrane (*BM*) × 55 600 (B) A fenestrated cap llary of the mod olus One of the fenestrae appears to contain a central knob (*arrow*) The pinocytot c ves cles show an attenuated membrane across the opening toward the basement membrane (*BM*) × 65 200 (C) Fenestrated cap llary of the mod olus The attenuated membranes (*ar ows*) are shown on the luminal s de of the vacuoles wh ch contain a substance s m lar to that of the capil lary lumen × 32 700

they are rather frequently seen along with other non fenestrated capillaries and arterioles They have a distinct feature of overall irregular thickness of the endothelium and are marked by the presence of tall microvilli and polyp like projections into the lumen (Fig 3 A) The fenestra are in the magnitude of 550 Å in diameter Some of the pinocytotic vesicles on the basal surface retain the attenuated membrane (Fig 4 B) Attenuated fenestra are also seen on the luminal side of the endothelium covering the large vacuoles (Fig 4 C) The endothelial cells do not contain filaments in bundle form They are rich in RNA and some of the rough endoplasmic reticula are dilated Vacuoles are frequently seen and pinocytotic vesicles are present in moderate number, more on the peripheral side even near the fenestra The basal surface is completely covered by the continuous basement membrane which averages 1 100 Å (range 990 to 1 270 Å) when measured from the outer margin of the dense part to the endothelial cell Pericytes make contact with the endothelial cell but do not extend over the fenestra The vessels are surrounded by a thin layer of fibrils and by the modiolar ells A preliminary study of the blood ves l within the cochlear nerve trunk shows that it is not fenestrated

The loose connective tissue of the modiolus is composed of blood vessels fibrocytes, unmyelinated nerve fibers, collagen fibers and fibrils The fibrocytes which constitute a major part of the tissue are different from those seen surrounding the major arteries These cells, modiolar cells, are very rich in large mitochondria, coated vesicles, and contain prominent large Golgi apparatus, many dilated rough endoplasmic reticula and numerous RNA particles in rosette form or in spiral, string like arrangement (Fig 5) Their nuclei are oval and large, and one or two centrioles or even short kinocilium may be seen near the nucleus The cytoplasm contains diffuse filaments and oval masses and sometimes rod shaped crystalloids (Fig 5 A) The main cell body often lies close to the fenestrated vessels, but more often their cell processes are attenuated and stacked in parallel where fascia occludens as well as desmosomes are frequently recognized Some of the modiolar cells appear in different phases of activity, their cytoplasm contains many less granulated endoplasmic reticula, large dense bodies and the Golgi network is less conspicuous (Fig 5 B) Unmyelinated nerve bundles are found in the midst of the modiolar cells away from the blood vessels (Fig 3 B), the presence of both adrenergic and cholinergic nerve fibers in the modiolus was reported recently by Wersall et al (1973)

The cochlear vessels after leaving the modiolar connective tissue enter into another twisting within the modiolar bone The arterioles seen in the bony channel are different from those seen among the modiolar cells The arterioles (16 μ) are rarely constricted to the degree seen in the modiolus The typical morphological appearance of the smooth muscle is less evident They lack the elastic layer as well as nerve fibers, however, nerve fibers are recognized by a fluorescence method described by Terayama et al (1966) The endothelial cells become flat, though some bulge is noted toward the lumen The cell organelles are decreased in number, but filaments in bundle form are clearly recognizable In the adventitia collagen fibers are not recognized and are occupied by densely packed fibrils and by a few fibrocytes

The arterioles in the bony interscalae partition and in the spiral ligament adjacent to the scala vestibuli are smaller but similar to those seen in the modiolar bone Thin smooth muscle cells or pericytes partly or completely surround the endothelial cells (cell height 0 3 μ) (Fig 6) The difference between smooth muscle cells and pericytes becomes less distinct as the size of the vessels is reduced In general, pericytes are thinner and do not surround the endothelial cells as much as the smooth muscle cells do, the dense foci at the central part of the cytoplasm are less con

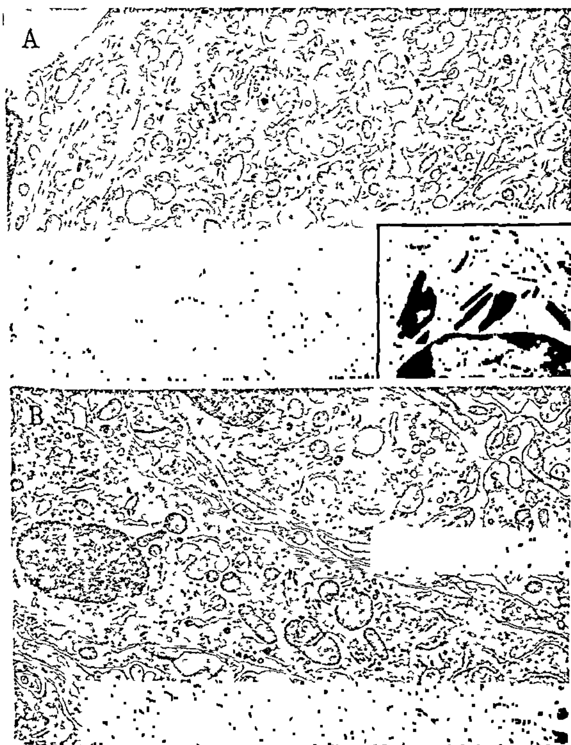


Fig 5 (A) Perivascular modiolar cells The cells contain numerous large mitochondria and their cell processes interdigitate considerably × 11 000 The inset shows modiolar cells containing elongated crystalloid substances and desmosomes between adjacent cells × 14 300 (B) The modiolar cells showing elongated and less granulated endoplasmic reticula vacuoles dense bodies and some mitochondria and scattered RNA particles × 11 000

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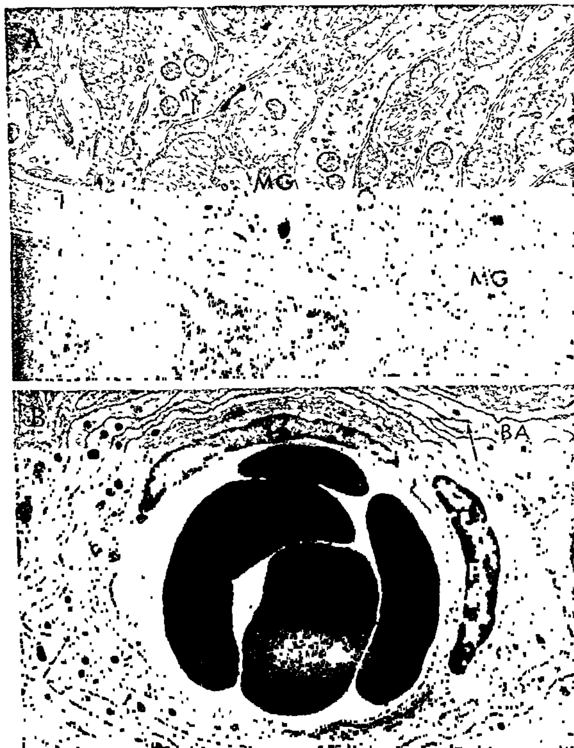



*Fig. 7* (A) A strial capillary abutted by marginal cells (*MG*) and intermediate cells (*I*). Pericyte (*P*) × 18 500. (B) The basal cells (*BA*) of the stria vascularis completely surround a capillary 8.3 μ. Note the lack of a perivascular fibrillar zone, and also numerous fascia occludentes which are indicated by dense patches between the cells (*arrows*). Pericyte (*P*) × 10 500.

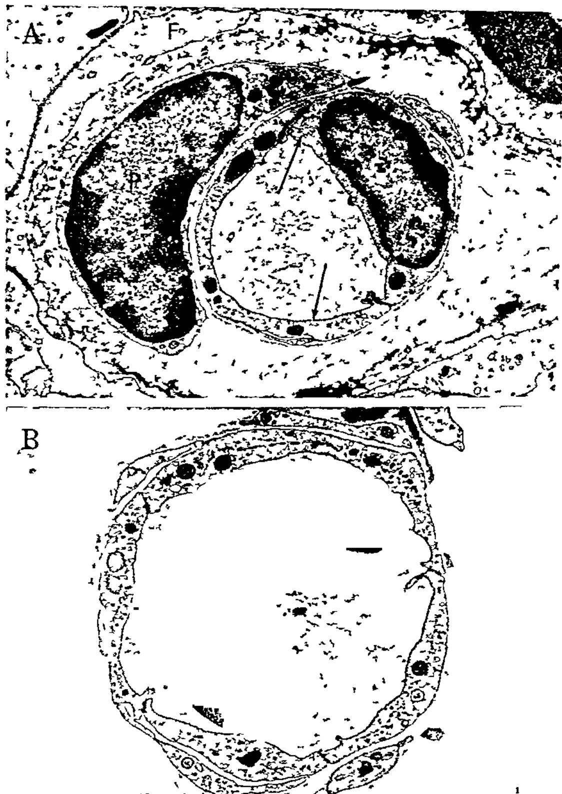



*Fig 8* (A) A capillary (4 2 μ) located in the spiral ligament adjacent to the scala vestibuli The endothelium contains filaments in bundle form (*arrows*) The vessel is surrounded by a loose fibrous zone and fibrocytes (*F*) Pericyte (*P*) × 11 500 (B) A capillary (5 5 μ) located in the spiral ligament adjacent to the scala tympani Intracellular filaments in bundle form are lacking Note a wide perivascular fluid space Pericyte (*P*) × 15 000

located among the epithelial cells (marginal cells) of the labyrinth The most obvious difference is their relation to adjacent cells, the vessels abut the marginal cells frequently and the intermediate cells randomly (Fig 7 A) The vessels retain pericytes throughout, no distinct smooth muscle characteristics are found Around the vessels are scant fibrils and, in a few instances, collagen fibers are demonstrable The basement membrane is continuous and may branch, but its thickness varies considerably (740 to 1 030 Å) with single or multiple layers Only adjacent to or at the vessels do the marginal cells retain the basement membrane which becomes continuous or fused together with that of the endothelial cells As the vessels enter or leave the stria vascularis, they are always tightly surrounded by the basal cells, which show numerous fasciae occludentes among themselves (Fig 7 B) The only intercellular pathway to and from the spiral ligament appears to be through the basement membrane of the vessels

Stria vessels vary considerably in size, the average diameter is 6 4 $\mu$ with a range of 3 5 to 12 7 $\mu$ The lumen generally retains a circular form, indicating very little con tracture of the wall The endothelial cell (height 0 1 to 0 5 $\mu$) is not fenestrated, and often shows marginal folds and some microvilli The tight junction may be seen near the luminal surface or in the mid portion of the junction or may be observed in three or four different segmental zones or may be con tinuous throughout The intracellular filamen tous bundles seen in the arteriolar vessels are rarely demonstrable The pinocytotic vesicles are found throughout the cytoplasm of the endothelial cells, though they tend to be more abundant at the basal portion Pericytes show numerous short processes, and the filaments within the cytoplasm are located on the side close to the endothelial cell along the cell curvature, while pinocytotic vesicles are located more away from the lumen The peri cytes form a tight junction with the endothelial cell by extending thin processes toward it, sometimes invaginating into it, also the main, broad part of the cell body extends and forms the tight junction The pericytes are completely surrounded by the basement membrane except at the endothelial cell junction and also sometimes near the marginal and intermediate cells The number of pericyte processes and endothelial cells in cross section of 57 strial vessels shows the endothelial cells number 4 2 and pericyte processes 6 3, which indicates that there are 1 5 times more pericyte processes Pericyte processes are always demonstrable no matter how small the vessel is, even in a capillary without endothelial cell junctions, which is seen in the squirrel monkey

The spiral ligament vessels seen behind the stria vascularis are generally small in diameter, averaging 5 8 $\mu$ with a range of 4 5 to 15 $\mu$ The cell organelles are not much different from those seen in the stria vascularis, although a fair number of the vessels, particularly arterio-venous arcades, contain intracellular filaments The basement membrane is thin, but the thickness varies considerably The vessels located deeper in the spiral ligament are surrounded by scant fibrils and fibrocytes, but toward the stria vascularis they are more tightly surrounded by fibrocytes Collagen fibers are not recognized The average number of endothelial cells in cross-section of 34 vessels is 4 3 and that of the pericyte processes is 6 4, which is very close to the figures obtained in the stria vascularis At the bifurcation point of capillaries in both the spiral ligament and the stria vascularis, pericyte processes are usually recognized but their structural details are not much different from those seen in the straight part of the vessels

The vessels of the spiral prominence (7 0 $\mu$) do not differ from those seen in the spiral ligament behind the stria vascularis Pinocytotic vesicles are more common at the basal surface The basement membrane is generally thinner than that of the stria vasc

The vessels are surrounded by a rather thick layer of fibrils which in turn are enclosed by tightly packed fibrocytes These spiral ligament cells (fibrocytes) are different from those seen in the deeper part of the spiral ligament, e g the mitochondria are often large and elongated and contain longitudinal cristae

Capillaries come close to the external sulcus cells twisting around the cell processes in the lower cochlear turns The vascular wall is usually interposed from the external sulcus cells by a thin layer of fibrils, though in some areas their basement membranes become continuous The perivascular fibrocytes are similar to those seen in the spiral prominence and to those of the Rhesus monkey (Takahashi & Kimura, 1970) Their morphology differs from the modiolar cells which are described earlier

The capillaries of the spiral ligament in the area adjacent to the scala tympani lack intracellular filaments in bundle form (Fig 8 B) However, in a few instances they are seen in larger venules The cell organelles found in the endothelial cells of this area are no different from those found in the endothelial cells of the scala vestibuli There is an indication that the number of endothelial cells in venules is smaller than that of arterioles of equal size (11 to 15 $\mu$) In the arteriole the profile of the primitive smooth muscle cells or pericytes is larger and often longer The number of endothelial cells and pericyte processes decreases from arterioles to capillaries and then increases in the venules The distribution pattern of pinocytotic vesicles in arterioles and venules are similar, they are located more at the basal surfaces The endothelial cell (cell height 0 2 $\mu$) of the venule tends to be thinner than that of the arteriole, the luminal surface shows less irregularity in the venule while in the arteriole the surface tends to bulge and the nuclei are often crenated There are no essential differences in the presence of microvilli and marginal folds in these two sides The basement membrane of the arteriole is thicker (600 to 1 344 Å) than that of the venule (477 to 625 Å) On the scala tympani side the vessels are surrounded by a wide zone of fluid and by scant fibrils and fibrocytes (Figs 8 B, 11 A) while on the arteriolar side there are more of these connective tissue elements

The blood vessels of the osseous spiral lamina are shown in two different zones one group within the bony channel and the other among the cochlear nerve fibers The vessels within the bony canals are small and lack nerve fibers in the perivascular zone The vessels among the cochlear nerve fibers are also small and their endothelial cells are surrounded by pericytes, fibrils and some collagen fibers. Unmyelinated nerve fibers are occasionally seen adjacent to these vessels, however, similar nerve fibers are seen among the cochlear nerve fibers Some of the unmyelinated nerve fibers are very small while others are large, both are randomly located The vessels of the limbus spiralis are similar to those of the spiral prominence and the external sulcus cells including the perivascular cells containing long mitochondria with longitudinally arranged cristae (Fig 9) At the tympanic lip unmyelinated nerve fibers are sometimes seen adjacent to the vessels, their vesicles generally lack the central core (Fig 10 A)

Blood vessels of the basilar membrane (vas spiralis) are small with an average diameter of 4 9 $\mu$ ranging from 3 2 to 7 0 $\mu$ (Fig 10 B) The outstanding feature of this blood vessel is its location, which is close to the organ of Corti There is nothing strikingly different about this vessel when compared with others In the endothelial cell intracellular filaments in bundle form are sometimes seen The vesicles are no more numerous than in other places and tend to occur more near the basal surface The endothelial cells are not fenestrated and are completely surrounded by the basement membrane Pericytes show numerous short processes and sometimes lack the basement membrane on the side away from the lumen The vas spiralis is usually surrounded by a thick layer of homogeneous



*Fig 9* A capillary (5 4 μ) in the limbus spiralis Note a large fibrous area and perivascular fibrocytes containing long mitochondria with longitudinal cristae similar to those seen in the spiral prominence and external sulcus vessels Pericyte (*P*) × 10 200

substance This substance is finely granular, though it looks like short filaments, it also forms irregular aggregates which appear as dark patches Outside the homogeneous layer are mesothelial cells partly or completely surrounding it and providing the appearance of perivascular channels (Hawkins, 1967) However, sometimes the mesothelial cells surround the vascular wall without an intervening layer of ground substance Nerve fibers are not recognized

The posterior spiral vein (Fig 11 B) and inferior cochlear vein (Figs 12 A, B) show similar morphological characteristics The endothelium is thin (0 3 to 0 8 μ) The diameter of the spiral vein is 79 μ at the basal turn, and that of the inferior cochlear vein near the scala tympani is slightly larger (average 92 μ) than near the cranial opening (average 83 μ) the significance of which is not clear at present

In the endothelial cells coated vesicles are many, and pinocytotic vesicles are located more often on the peripheral side (Fig 12 A) Bundles of intracellular filaments are sometimes seen in the endothelial cells of the inferior cochlear vein (Fig 12 B) The luminal surfaces show occasional prominent marginal folds and villous projections, and the basal surfaces reveal some cytoplasmic extensions The tight junction is often very short, but the cell junction may be wide throughout (125 Å) The basement membrane is thin and continuous, though in some areas a discontinuity is noted Pericytes or primitive smooth muscle cells (Rhodin, 1968) are widely spaced, and the processes appear to overlap in some areas, the basement membrane is often lacking toward the endothelial cells More peripherally, there is a wide space with loosely organized fibrils and a small number of fi

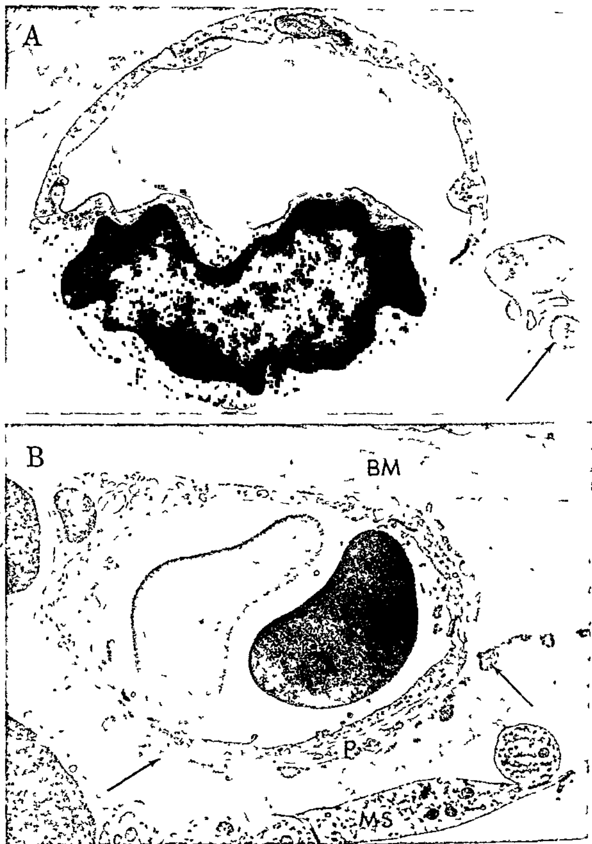



*Fig 10* (A) A capillary (5 3 μ) at the tympanic lip of the limbus spiralis Note some vesicles in the unmyelinated nerve fibers partly covered by the Schwann cell (*arrow*) Pericyte (*P*) × 21 300 (B) The vas spiralis (5 6 μ) below the basilar membrane (*BM*) Note the homogeneous substance around the vessels dense patches (*arrows*) and mesothelial cells (*MS*) Pericyte (*P*) × 11 500




*Fig 11* (A) A collecting venule (12 $\mu$ diameter) of the spiral ligament adjacent to the scala tympani. Note thin vascular wall and a wide fluid space on the outside Compare with the arteriole in Fig 6 Pericyte (*P*) × 10 000 (B) Vascular wall of the posterior spiral vein within the bony canal from the basal turn The endothelium is thin, pericyte processes (*arrows*) are short and widely spaced, and the connective tissue is scant. × 14 000.

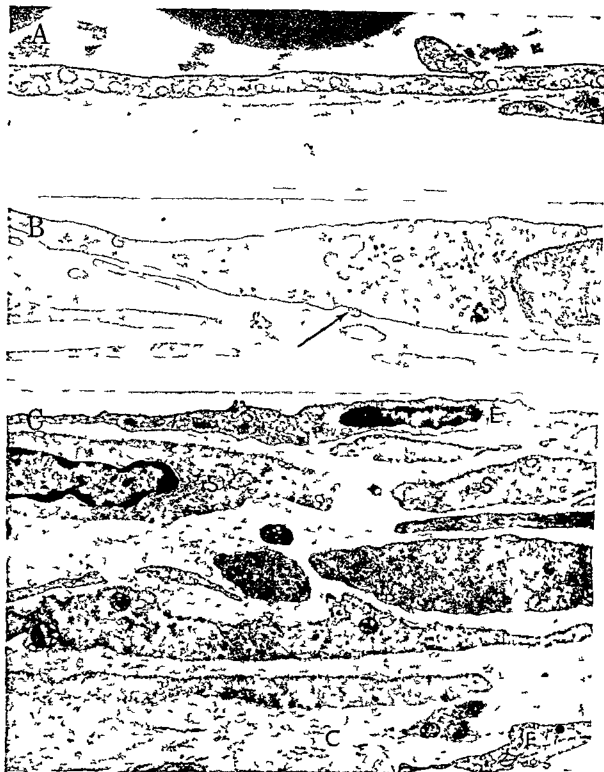



*Fig 12* (A) The endothelium of the inferior cochlear vein within the bony canal Note numerous pinocytotic vesicles at the basal surface and absence of pericytes ×34 000 (B) The endothelium of the inferior cochlear vein showing filaments in bundle form Pericyte (*arrow*) ×17 600 (C) Vascular wall of the inferior petrosal sinus showing the endothelium (*E*) smooth muscle cells (*S*) fibrocytes (*F*) and collagen fibers (*C*) ×11 000

brocytes The inferior cochlear vein in the dura shows pericytes or primitive smooth muscle cells still widely spaced, but numerous collagen fibers and a few unmyelinated nerve fibers begin to appear Smooth muscle cells, in layers varying from one to six, are shown in the inferior petrosal sinus (Fig 12 C) They make contact with the endothelial cells within which filaments are scarce, and are not arranged in bundle form

## DISCUSSION

Clear morphological differences are noted between the small blood vessels of the modiolus and those of the lateral wall of the membranous labyrinth The smooth muscle cells of the modiolar arterioles are similar to those of the peripheral vascular bed (Rhodin, 1967), but those of the radiating arterioles of the spiral ligament are flat, widely spaced and resemble pericytes The lack of a nerve supply and the lack of typical smooth muscle characteristics suggest that the ability of these vessels to contract is rather limited These vessels do not contract when the cervical sympathetic nerve is stimulated nor if adrenalin chloride is locally or systemically applied to these vessels (Perlman & Kimura, 1955) The major control of blood flow appears to be accomplished at the modiolus

The fenestrated vessels seen in the modiolar connective tissue are similar to those described earlier in the endolymphatic sac (Lundquist et al, 1964) The fenestrated vessels are presumed to be related to rapid transfer of fluid and solutes, and are reviewed extensively by Majno (1965) The fenestrated vessels seen in the modiolus are probably a branch of the main cochlear artery and are not expected to reach the membranous labyrinth The cells surrounding these fenestrated vessels show an unusually high protein metabolism, they are compared to the choroid plexus in producing cerebrospinal fluid, and are named the cochlear plexus by Balogh & Koburg (1965) The present study shows that the modiolar cells (cells of the cochlear plexus) are essentially fibrocytes, while the cells of the choroid plexus are epithelial cells with a distinct basement membrane The modiolar cells may serve not only to support the vessels but also to participate in production or modification of modiolar fluid A brief ultrastructural description of the modiolar cells is provided earlier by Mootz & Musebeck (1970) It is well known that the cochlear vessels coil or twist within the connective tissue and also in the modiolar bone (glomeruli of Schwalbe, Hawkins, 1967) before becoming a straight line at the level of the radiating arterioles (Smith, 1951) At the coiled portion of the vessels, energy is presumably dissipated in reducing the pulsatile movement of blood The fenestrated vessels, which are apparently branches of these coiled vessels, may provide rapid transfer of fluid and may even play a role in reducing blood pressure These vessels are undoubtedly the major source of fluid surrounding the neural elements of the modiolus

Along the course of the cochlear blood vessels some differences in the cytoarchitecture of the endothelial cells are noted One variance is intracellular filaments in bundle form These filaments are more prevalent in the vessels in which blood flow is very fast Their arrangement may aid in propagating the movement of blood in the longitudinal direction by stretching and relaxing These endothelial filaments are common in other vessels (Bensch et al, 1964, Giacomelli et al, 1970), and have been considered to play a role in supportive (Cecio 1967) as well as contractile function (Fawcett, 1959) Contraction of the endothelial cell is known to occur even without pericyte contraction when histamine is applied (Majno et al, 1969)

Pinocytotic vesicles are common throughout the vascular channels Arterioles and venules show no essential difference in vesicle distribution Both of them show more vesicles at the basal surface Their presence is apparently not associated with the gas ex-

change of blood corpuscles, but appears to be closely associated with the cells involving active fluid transfer They may serve to maintain fluid balance between blood and the interstitial space The vesicles are generally considered vehicles of fluid or solute transfer across the cell, and/or they may carry some substance for intracellular use (Palade, 1953, Fawcett, 1966) Duvall et al (1971) demonstrated the passage of horseradish peroxidase through the strial endothelium by pinocytotic vesicles, not through the endothelium of the perilymphatic vessels This is interpreted by Wersall et al (1973) to be due to the high density of vesicles in the strial capillaries

The basement membrane is thicker on the arteriole side in comparison to the venule Also it is often thick in the stria vascularis and in the fenestrated capillaries of the modiolus Aging is apparently one of the factors involved in the increase in thickness (Gersh & Catchpole, 1949, Kurtz & Feldman, 1962) A very thick basement membrane is reported earlier in the stria vascularis of old humans (Kimura & Schuknecht, 1970) A correlation between blood pressure and the thickness of the basement membrane has been reported by Pease (1960) The thickest basement membrane is found in the renal glomerulus and in the renal capillaries where the capillaries are fenestrated and the blood pressure is high (Majno 1965) The marginal cells of the stria vascularis are often compared to renal epithelium in their possible function of active fluid transport (Smith, 1957) However, the blood pressure within the strial capillaries is not known The basement membrane is considered to be a product of the endothelial cells and to act as a selective filter, mechanical support for the endothelial cells, an anchorage for connective tissue, and a guide for regenerating vessels (Majno, 1965)

It is of interest to note that the ratio of pericyte processes to endothelial cells is just about the same in both the stria and the spiral ligament Clear evidence of pericyte contraction has been lacking or the magnitude of contraction is too difficult to determine under the light microscope Under physiologic conditions neither mechanical nor drug stimulus is capable of inducing a visible change in the caliber of the vessels on the lateral membranous wall (Perlman & Kimura, 1955, Perlman et al, 1963) However, since these cells contain filaments similar to those seen in smooth muscle, they are considered capable of contraction (Rhodin, 1968) If they contract in the cochlea, the effectiveness must be greater in the vessels located adjacent to the scala vestibuli, and very little in the stria vascularis and the scala tympani side

Except in the stria vascularis, adventitial cells are all fibrocytes or modifications of fibrocytes These perivascular cells differ in morphology in different locations and are given specific names to identify them The intercellular substances also differ, though their differences cannot be attributed to any specific cytoplasmic substance The most remarkable differences are a sudden disappearance of collagen fibers (427 Å) in the membranous part of the labyrinth, an enormous increase in the ground substance (amorphous material) at the basilar membrane and an almost complete lack of fibrils and collagen fibers in the stria vascularis The close arrangement of the marginal cells to the capillaries is not found among the vestibular secretory cells (dark cells) which are the counterpart of the strial marginal cells, both of these cells are presumed to play a major role in maintaining a high potassium level in the endolymph (Kimura, 1969) An increase in endolymph pressure, however, may affect the strial capillaries more adversely because of this anatomical arrangement and the tight delineation established by the basal cells The strial atrophy seen in the endolymphatic hydrops may be partly accounted for by this mechanism (Kimura, 1967) The close relationship between marginal cells and capillaries undoubtedly facilitates a turnover of endolymph

## ZUSAMMENFASSUNG

Die Ultrastruktur der cochlearen Blutgefasse wurde von den Arterien bis zu den Venen studiert Einige wichtige Unterschiede zwischen den kleineren Gefassen des Modiolus und den Gefassen des membranosen Labyrinths wurden gefunden Morphologische Befunde sprechen dafür, dass die Blutstromhauptkontrolle fur das Cortische Organ im Modiolus liegt Kapillaren mit Fenstern wurden zwischen den Modioluszellen, die ungewohnlich reich an Zellorganellen sind, gefunden Diese Gefasse bilden wahrscheinlich die Hauptquelle der Modiolusflüssigkeit und könnten dazu dienen, den Blutdruck und die pulsierenden Bewegungen der Blutgefasse zu reduzieren Intrazellulare Filamente, die in Bündeln gruppiert sind, wurden oft in den endothelialen Zellen der Arterien, Arteriolen und arteriovenosen Arkaden gefunden Sie sind meistens in Gefassen mit sehr schnellem Blutstrom vorhanden Die Kapillaren standen in Kontakt mit marginalen, intermedialen und basalen Zellen Um die Venülen und die Venae cochlearis inferior fanden sich weit auseinander liegende Perizyten und seltsame Fibrozyten Es waren keine Nervenfasern in der direkten Umgebung der Gefasse des membranosen Labyrinths vorhanden, mit Ausnahme der tympanischen Lippe des Limbus spiralis

## REFERENCES


Balogh, K & Koburg, E 1965 Der Plexus cochlearis *Arch Ohr Nas Kehlkopfheilk 185* 638

Bennett, H S, Luft, J H & Hampton, J G 1959 Morphological classification of vertebrate blood capillaries *Amer J Physiol 196*, 381

Bensch, K G, Gordon, G B & Miller, L 1964 Fibrillar structures resembling leiomyofibrils in endothelial cells of mammalian pulmonary blood vessels *Z Zellforsch 63*, 759

Cecio, A 1967 Ultrastructural features of cytofilaments in mammalian endothelial cells *Z Zellforsch 83*, 40

Duvall, A J, III, Quick, C A & Sutherland, C. R 1971 Horseradish peroxidase in the lateral cochlear wall *Arch Otolaryng* (Chic ) *93* 304

Farquhar, M G 1961 Fine structure and function in capillaries of the anterior pituitary gland *Angiology 12*, 270

Fawcett, D W 1959 The fine structure of capillaries, arterioles and small arteries In *The microcirculation* (ed S R M Reynolds & B W Zweifach), University of Illinois Press, Urbana

— 1963 Comparative observations on the fine structure of blood capillaries In *The peripheral blood vessels* (ed J L Orbison & D Smith), p 17 its significance in tissue injury, disease and growth *Amer J Anat 85*, 457

Giacomelli, F, Wiener, J & Spiro, D 1970 Cross-striated arrays of filaments in endothelium *J Cell Biol 45*, 188

Hawkins, J E, Jr 1967 Vascular patterns of the membranous labyrinth *Third symposium on the role of the vestibular labyrinth in space exploration*, p 241 NASA SP 152, National Aeronautics and Space Administration, Washington, D C

Hinojosa, R & Rodriguez Echandia, E L 1966 The fine structure of the stria vascularis of the cat inner ear *Amer J Anat 118*, 631

Iurato, S 1967 Basement membrane and connective membrane In *Submicroscopic structure of the inner ear* (ed S Iurato), Chapter IV, p 54 Pergamon Press, New York

Karnovsky, M J 1965 A formaldehyde glutaraldehyde fixative of high osmolality for use in electron microscopy *J Cell Biol 27*, 137A

Kimura, R S 1967 Experimental blockage of the endolymphatic duct and sac and its effect on the inner ear of the guinea pig *Ann Otol 76*, 664

— 1969 Distribution, structure and function of dark cells in the vestibular labyrinth *Ann Otol 78*, 542

Kimura, R S & Schuknecht, H F 1970 The ultrastructure of the human stria vascularis Part I *Acta Otolaryng* (Stockh ) *69*, 415

Kurtz, S M & Feldman, J D 1962 Experimental studies on the formation of the glomerular basement membrane *J Ultrastruct Res 6*, 19

Luft, J H 1965 The ultrastructural basis of capillary permeability In *The inflammatory process* (ed B W Zweifach, L. Grant & R T McCluskey), p 121 Academic Press, New York

Lundquist, P G, Kimura, R & Wersall, J 1964 Ultrastructural organization of the epithelial lining in the endolymphatic duct and sac in the guinea pig *Acta Otolaryng* (Stockh ) *57*, 65

Majno, G 1965 Ultrastructure of the vascular membrane In *Handbook of physiology* (ed W F Hamilton & P Dow), Section 2, Circulation, vol III, p 2293 American Physiological Society, Washington, D C

Majno, G, Shea, S M & Leventhal, M 1969 Endothelial contraction induced by histamine-type mediators *J Cell Biol 42*, 647

Mootz, W & Müsebeck, K. 1970 Die Ultrastruktur des Plexus cochlearis *Arch Ohr Nas Kehlkopfheilk 196*, 301

Palade, G E 1953 The fine structure of blood capillaries *J Appl Physics 24*, 1424

— 1961 Blood capillaries of the heart and other organs *Circulation 24*, 368

Pease, D C 1960 The basement membrane substratum of histological order and complexity In *Vierter internationaler Kongress fur Elektronen-Mikroscopie* (ed W Bargmann), p 139 Springer-Verlag, Berlin

Perlman, H B & Kimura, R S 1955 Observations

on the living blood vessels of the cochlea *Ann Otol 64* 1176
Perlman H B Tsunoo M & Spence A 1963 Cochlear blood flow and function effect of pressor agents *Acta Otolaryng* (Stockh) *56* 587
Rhodin J A G 1967 The ultrastructure of mammalian arterioles and precapillary sphincters *J Ultrastruct Res 18* 181
— 1968 Ultrastructure of mammalian venous capillaries venules and small collecting veins *J Ultrastruct Res 25* 452
Smith C A 1951 Capillary areas of the cochlea in the guinea pig *Laryngoscope 61* 1073
— 1957 Structure of the stria vascularis and the spiral prominence *Ann Otol 66* 521
Takahashi T & Kimura R S 1970 The ultrastructure of the spiral ligament in the Rhesus monkey *Acta Otolaryng* (Stockh) *69* 46
Terayama Y Holz E & Beck C 1966 Adrenergic innervation of the cochlea *Ann Otol 75* 69
Wersall J Densert O & Lundquist P G 1973 Studies on fine structure of inner ear vessels In *Vascular disorders and hearing defects* (ed A J D de Lorenzo) University Park Press Baltimore

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# STAINING OF HAIR CELL NUCLEI BY A FLUORESCENT DERIVATIVE OF HEMICHOLINIUM-3

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*Abstract* The uptake of terphenyl HC-3, a fluorescent derivative of hemicholinium 3, by cochlear hair cells was investigated in normal and damaged guinea pig cochleas The uptake of terphenyl HC 3 by hair cell nuclei is markedly reduced in Kanamycin and noise-induced cochlear damage This reduction of terphenyl HC 3 uptake occurs before the appearance of the classic distortions in the gross morphology of the hair cells resulting from such damage

Hemicholinium-3 (HC-3) is an agent which competitively inhibits cholinergic neurons from taking up choline and which thereby causes a reduction in the ability of such neurons to synthesize acetylcholine (Ach) (Schueler, 1960) This action has been of use in determining whether a given set of neurons are cholinergic or not For instance, application of HC-3 has been shown to cause a steady diminution in the inhibitory influence of the olivo-cochlear bundle (OCB) thus suggesting that the OCB is cholinergic (Guth & Amaro, 1969)

Many derivatives of HC-3 have been synthesized in hope of delineating its active moieties and improving its actions Among these derivatives is terphenyl-HC 3 (THC-3) a fluorescent compound Haarstad (1973) first thought to make use of THC-3's fluorescent property as a histochemical agent with some specificity for cholinergic endings, the sites where choline tends to be most avidly taken up for the synthesis of Ach

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In the present work, THC-3 was applied to the organ of Corti in the hope that it would associate primarily with the only structures in the cochlea known to be cholinergic, the olivo-cochlear bundle (OCB) endings

## METHODS

Guinea pigs of both sexes weighing between 200 and 500 grams were used Fluorescent staining of the organ of Corti was obtained in the following manner After sacrificing the animals with overdoses of pentobarbital i p, their temporal bones were removed, bullae opened and cochleae exposed The "cap" of the cochlea and a portion of the base near the round window were cracked open and 2% $O_sO_4$ was allowed to flow through the cochlea The cochleae remained submerged in the $O_sO_4$ for at least 2 min before being rinsed in three changes of physiological saline solution THC-3 solution containing THC-3 $5\times10^{-4}$ M and choline chloride $5\times10^{-2}$ M (the choline was included to reduce background fluorescence, according to Haarstad, 1973), made just before use and continually warmed to keep the THC-3 in solution was then forced through the basal and apical ends of the cochlea Cochleae were then submerged in the THC-3 solution for 10 min before being rinsed twice in physiological saline solution Dissection and mounting of the basilar membrane was performed in 50% glycerol These surface preparations were examined immediately by means of ultraviolet and phase-contrast microscopy If not examined immedi-

ately the fluorescence tended to "fade" in time This fading is not seen in permanently-mounted sections from the central nervous system, so it is surmised that the THC-3 gradually diffuses out into the glycerol in which the basilar membrane pieces are mounted The ultraviolet examination was made using a Leitz Ortholux UV microscope with a BG12 5 mm filter and a K510 suppression filter in dark field

Three guinea pigs were treated with Kanamycin sulfate to induce hair cell damage One received 400 mg/kg i p for 8 days and then was allowed 7 drug-free days before sacrifice The other two guinea pigs also received 400 mg/kg i p for 8 days and no drugs for a week, but because both animals still had a vigorous Preyer reflex, drug treatment was reinstituted One of these two animals received 400 mg/kg i p for a further 6 days, followed by 30 drug free days before sacrifice The third animal received 400 mg/kg for 8 days then no drug for 7 days, then 400 mg/kg for 6 days, then 600 mg/kg for 3 days and no drug for a final 14 days All cochleae of these animals were treated as described above Control animals were sacrificed along with the Kanamycin-treated animals

Noise trauma was employed in 9 guinea pigs These animals were all anaesthetized with pentobarbital (30 mg/kg i p ) The middle ear on one side (control side) was then destroyed and the animals placed in a box for varying periods of time alongside a firebell which produced noise at 115 dB SPL These animals were either kept for several days or sacrificed immediately and their cochleae treated as described above

## RESULTS

The arresting pattern of fluorescent dots clearly suggests association with the hair cells (Fig 1) At first, this seemed to confirm the hypothesis that THC-3 was being taken up by the cholinergic OCB endings That first impression was further strengthened by the observation that the intensity and distribution of fluorescence tended to parallel the distribution of OCB endings in the cochlea That is, the outer rows of hair cells in the basal end of the cochlea stained most intensely whereas in the middle and apical portions of the basilar membrane both inner and outer hair cells fluoresced but less intensely than at the basal end

However, when the size and shape of the fluorescent dots was examined more closely it seemed clear that they were more likely associated with hair cell nuclei The fluorescent dots are almost perfectly round and have a diameter nearly the same as outer hair cell nuclei Furthermore, when the distances between nuclei within rows and between rows is measured they coincide almost exactly with these same distances measured between fluorescent dots

To test the possible association of the "dots" with the hair cells it was decided to cause hair cell destruction by treating guinea pigs with ototoxic doses of Kanamycin In all 3 treated animals no orderly rows of fluorescent "dots" appeared This was so in spite of the fact that many hair cells and their nuclei appeared to be intact (Figs 2 & 3) although much hair cell destruction and scarring was seen This unexpected result, i e the lack of THC-3 uptake by apparently intact hair cell nuclei, was set aside until the results of another experimental stratagem, could be studied It was decided to attempt to disrupt the cochlear hair cells by using high intensity noise prior to application of THC-3 Once again, although most of the hair cells and their nuclei appeared to be intact when viewed under phase-contrast microscopy (Fig 4), either no orderly rows of fluorescent dots were seen or there was a marked difference in fluorescent intensity between the control (middle ear destroyed prior to noise trauma) and treated cochleae That THC-3 still gains access to the organ of Corti is witnessed by the fact that other cochlear structures presumably supporting cell nuclei which appear elliptical rather than round and not in orderly rows, continue to take it up and fluoresce (Fig 5) The conclusion drawn from these results is that the ability of the hair cells to take up THC-3 is very sensitive to Kanamycin and noise induced cochlear damage In fact, the weakening or actual loss of fluorescent staining

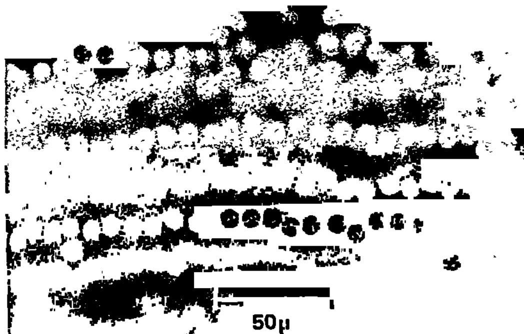



*Fig 1.* Ultraviolet light photomicrograph of guinea pig basilar membrane, the basal turn, showing the regular rows of round fluorescent dots produced by exposing osmicated basilar membrane to THC-3




*Fig 2* White light phase contrast photomicrograph of hair cells from the middle turn of Kanamycin-treated guinea pigs

*Fig 3* White light phase contrast photomicrograph of hair cell nuclei from the same region as *Fig 2*.

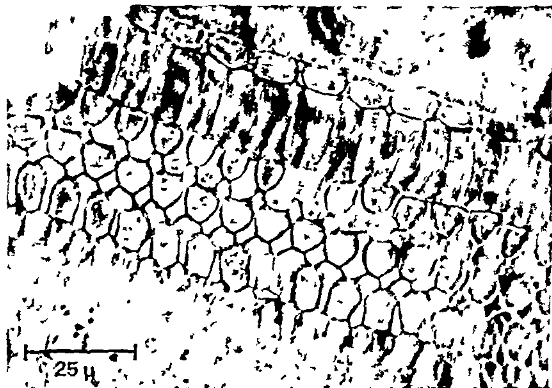



*Fig 4* White light phase contrast photomicrograph of hair cells from middle turn of the cochlea The guinea pig in this case was subjected to 115 dB S P L bell no… for 60 min Middle ear intact

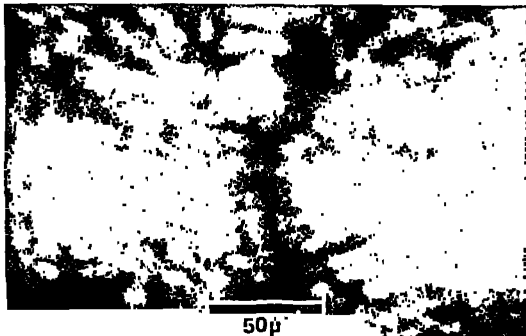



*Fig 5* Ultraviolet light photomicrograph of a similar region as Fig 4 Middle ear intact

in response to noise or Kanamycin occurs before the now-classic gross distortions of cochlear morphology such as hair cell nuclear swelling, and breakdown, and actual scarring of the hair cells The main purpose of this paper is, in fact, to apprise otologic researchers of this fact and of its possible use as a tool in further otologic research

The particular cochlear distribution of the fluorescence is of interest in that it appears to parallel the well-known pattern of damage induced by noise or ototoxic agents as well as the olivo-cochlear bundle endings

The authors propose next to study whether the uptake of THC-3 by hair cell nuclei is related to the uptake of choline by these structures

## ACKNOWLEDGMENT

The authors gratefully acknowledge the outstanding assistance of Mr T L Hayward

## ZUSAMMENFASSUNG

Die Aufnahme von Terphenyl HC 3, einem fluoreszierenden Derivat von Hemicholinium-3, durch die Haarzellen im Cortischen Organ des Meerschweinchens wurde untersucht, und zwar sowohl in normalen als auch in geschädigten Cochleae Die Aufnahme von Terphenyl-HC-3 durch den Zellkern ist nach Beschädigung der Cochlea durch Lärm oder Kanamycin bedeutend reduziert Diese Reduktion tritt vor dem Auftreten der klassischen Verzerrung in der Morphologie der Haarzelle ein, welche durch solch Beschädigung verursacht wird

## REFERENCES

Guth, P S & Amaro, J 1969 A possible cholinergic link in olivo-cochlear inhibition *Int J Neuropharm* *8*, 49

Haarstad, V B 1973 Personal communication

Schueler, F W 1960 The mechanism of action of the hemicholiniums *Int Rev Neurobiol 2*, 77

*Note added in prof* Since submission of this manuscript a paper has appeared (Goldstein, A J 1973 Permeability of the organ of Corti *Ann Otol 82*, 166) in which a similar lack of staining of cochlear hair cells following sound stimulation was reported The fluorescent stain employed was fluorescein diacetate The dissimilarity of the two stains employed suggests that the change induced by sound affects some general process such as permeability as Goldstein suggests

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# ZUR REIZINTERVALL-ABHÄNGIGKEIT AKUSTISCH EVOZIERTER POTENTIALE

E Stürzebecher, H Wagner und H -J. Gerhardt

*Aus der Hals-Nasen-Ohren Klinik der Humboldt-Universität (Charite), Berlin, DDR*

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*Abstrakt* Die Abhangigkeit der $N_1$-$P_2$-Amplitude und der Latenzen akustisch evozierter Potentiale vom Reizintervall (0,2 s 10 s) wurde bei den Reizstarken 20, 30, 50, 70 und 90 dB u S gemessen Im untersuchten Intervallbereich erreichen die Amplituden (außer der 20-dB-Kurve) noch nicht ihren Hochstwert Jedoch zeigt eine Extrapolation der Kurven uber 10 s hinaus, daß bereits bei 10 s mindestens 90 % der Maximal Amplitude erreicht werden Die prozentuale Amplitudenabnahme bei kürzeren Intervallen (bezogen auf den Wert bei 10 s) ist fur alle Reizstärken etwa gleich Durch Umzeichnen der Amplituden-Meßwerte wird eine Reizstärke — Erregungskennlinienschar mit der Intervalldauer als Parameter erhalten Mit der Intervalldauer steigen die $N_1$- und $P_2$ Latenzen signifikant an, $t_{LP_2}$ starker als $t_{LN_1}$ Bei kurzen Intervallen durchlaufen beide Kurvenscharen ein Minimum Eine Änderung des Schallpegels bewirkt m wesentlichen nur eine Parallelverschiebung der rven, deren Ausmaß eine Funktion des Schallpegels ist Infolge dieses Verhaltens ist die Latenzdifferenz der Antworten bei kurzen und bei langen Intervallen nahezu unabhangig von der Reizstärke

Die Tatsache, daß die $N_1$-$P_2$-Amplitude des akustisch evozierten Potentials (AEP) stark von der Reizintervall-Dauer abhängt, ist allgemein bekannt Der funktionelle Zusammenhang zwischen Intervalldauer und AEP-Amplitude sowie der Einfluß der Intervalldauer auf die Form der Kennlinie (AEP-Amplitude $= f$ ($L_{Aud}$ /dB u S ))[1] werden jedoch nur in wenigen Arbeiten angegeben und erstrecken sich nicht über den gesamten interessierenden Intensitäts- bzw Intervallbereich Davis et al (1966) haben die Abhängigkeit der $N_1$-$P_2$-Amplitude des AEP vom Reizintervall für Tonpips (gefilterte Clicks, 1 200 Hz, 85 dB ISO) angegeben Nelson & Lassman (1968) haben den Zusammenhang zwischen Stimulus-Intervall und AEP-Amplitude mit 20 ms Tonbursts bei einer Reizstärke (60 dB) fur mehrere Frequenzen gemessen Beide Ergebnisse sind wegen der Unterschiede in der Reizart nicht direkt vergleichbar Gjerdingen & Tomsic (1970) haben die Recovery Funktionen der Potentiale, die durch Tone, elektrische Schocks, Vibration und Lichtreize evoziert wurden, verglichen

Ziel unserer Arbeit zu dieser Fragestellung war es, die Abhängigkeit der AEP-Amplituden und -Latenzen von der Reizintervalldauer bei einer Frequenz (1 000 Hz) über den gesamten interessierenden Schallpegel-Bereich zu untersuchen

## METHODIK

Die Untersuchungen wurden in einer schallisolierten Kammer durchgeführt Als Versuchspersonen (VP) dienten 10 normalhörende Studenten im Alter von 20–25 Jahren Die Elektrodenlage war Vertex gegen Mastoid, Erdung erfolgte am Ohrläppchen Als Reizfrequenz wurden 1 000 Hz gewählt, die Reizdauer war 500 ms mit 25 ms Anstiegs- und Abfallzeit (Die Anstiegszeit ist in den 500 ms enthalten) Das verwendete Audiometer der Firma PAR ist nach ISO-Norm kalibriert Die Mittelung wurde mit einem Averager vom Typ DL 102A der Firma DATALAB durchgeführt, dessen

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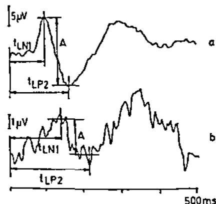



*Abb 1* Demonstration der Methodik der Potentialausmessung (*a*) Potential mit geringem Störsignalanteil (gemittelt bei 70 dB u S und 2 s ISI) (*b*) Potential mit hohem Storsignalanteil (gemittelt bei 20 dB u S und 1 s ISI) *Anzahl* der Mittelungsschritte in beiden Fallen *n* = 64

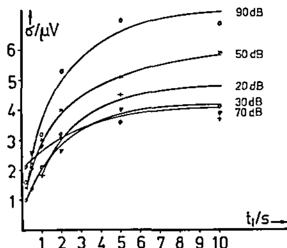



*Abb 3* Standardabweichung der AEP Amplituden in Abhangigkeit von der Pausendauer zwischen den Reizen

besonderer Vorteil die Möglichkeit einer automatischen Artefact-Unterdrückung ist Als EEG-Verstärker wurde der Verstärker Typ 113 der Firma PAR mit einem Eingangswiderstand von 100 MΩ verwendet Die obere Grenzfrequenz war 30 Hz, die untere 0,3 Hz

Wahrend der Untersuchung lasen die VP Texte nach eigener Wahl Als Amplitude des AEP wird die Differenz zwischen $N_1$ und $P_2$ bezeichnet Da bei geringen Reizstärken und kurzen Reiz Intervallen das Signal–Stör Verhaltnis ungünstiger ist, ergibt sich als Mittelungsergebnis bei den gewählten 64 Mittelungsschritten u U kein glatter Kurvenzug Die dabei angewandte Methodik der Ausmessung veranschaulicht Abb 1

Bei jeder VP wurde die Intervall Abhängigkeit bei den Audiometer-Reizstärken 20, 30 und 50 dB ü S je 4mal, bei 70 und 90 dB ü S je 3mal *gemessen Alle Meßpunkte jeder Kurve* sind daher Mittelwerte von jeweils 40 bzw 30 Einzelpotentialen (10 VP) Gepruft wurde bei den Interstimulus-Intervallen (ISI) 0,2, 0,5, 1, 2, 5, 10 s Als ISI wird die Dauer der Pause zwischen zwei aufeinanderfolgenden Reizen bezeichnet

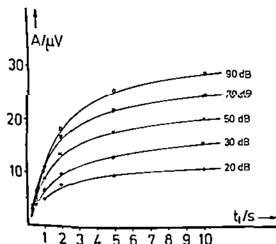



*Abb 2* Abhängigkeit der $N_1$ $P_2$-Amplitude des AEP von der Pausendauer zwischen den Reizen mit dem Schallpegel als Parameter (Standardabweichungen siehe Abb 3)

## ERGEBNISSE

Abb 2 zeigt die ermittelte Abhängigkeit der AEP-Amplitude von der Dauer der Pause zwischen den Reizen bei 90, 70, 50, 30 und 20 dB u S Zur besseren Übersichtlichkeit wurde die fur alle Meßpunkte berechnete Standardabweichung $\sigma$[1] gesondert in Abb 3 dargestellt

Aus Abb 2 ist ersichtlich, daß auch bei einem ISI von 10 s die bei dem jeweiligen Schallpegel

[1] Obwohl es sich hier um die Standardabweichung in der Stichprobe und nicht in der Grundgesamtheit handelt wurde statt *s* die Bezeichnung σ gewählt, um eine Verwechslung mit der Maßeinheit der Intervalldauer (Sekunde) zu vermeiden

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Fruhstorfer et al (1969), Rothman et al (1970) und Ruhm (1971) fanden ebenfalls, daß 10 s Intervalle bei akustischer Reizung ausreichend sind fur vollständige Erholung

Nelson & Lassman (1968) fanden einen linearen Zusammenhang zwischen der AEP-Amplitude und dem logarithmisch aufgetragenen Reiz Intervall im Bereich 0,25–6 s Dieses Ergebnis wird durch die vorliegenden Messungen nicht bestätigt Erklärt werden kann dieser Unterschied und auch die bedeutend kleineren Amplituden in der genannten Arbeit eventuell durch den wesentlich kürzeren Ton-Reiz (20 ms), den die Autoren verwendeten Die prozentuale Amplitudenverminderung bei kürzeren Intervallen (bezogen auf den Wert bei 10 s) ist für alle gemessenen Reizstärken etwa gleich Webster (1972) fand ebenfalls keine Differenzen zwischen der Amplitudenabnahme bei hohen und bei niedrigen Reizstärken Da er auch keine Dishabituation nachweisen konnte, schlußfolgert er, daß die Amplitudenminderung akustisch evozierter Potentiale bei kurzen Reiz-Intervallen im wesentlichen nicht durch Habituation bewirkt wird

Davis et al (1966) konnten keine Latenzänderungen akustisch evozierter Potentiale in Abhängigkeit vom ISI nachweisen Nelson & Lassman (1968) gaben nur für $P_2$ und $N_2$ eine signifikante Latenzverlängerung bei längeren Intervallen an Die in der vorliegenden Arbeit dargestellte signifikante Anderung (0,5%-Niveau) auch der Latenz von $N_1$ wird durch Ergebnisse von Karnahl & Benning (1972) bestätigt Die Autoren haben die Abhängigkeit der Latenz von der Reizstärke bei zwei unterschiedlichen Reiz-Intervallen gemessen (1,3 s–3,6 s Intervalle = „adaptierter Zustand", 5 s 20 s Intervalle = „nichtadaptierter Zustand") Die mittleren Intervalle der zufallsverteilten Reizfolgen sind leider nicht angegeben Die Autoren fanden für den nichtadaptierten Zustand signifikant längere $P_1$-, $N_1$- und $P_2$-Latenzen Die Latenzdifferenz adaptiert–nichtadaptiert ist für $P_1$ am kleinsten und fur alle 3 Wellen nahezu unabhängig vom Schallpegel

Zeichnet man für die Parameter $t_i = 10$ s und $t_i = 2$ s mit den zu diesen Intervallen gehorenden Meßwerten aus Abb 4 die Verläufe $L_{N1} = f(L_{Aud.})$ und $L_{P2} = f(L_{Aud.})$, so erhält man qualitativ dieselben Ergebnisse Ein quantitativer Vergleich ist nicht möglich wegen der fehlenden Angabe der mittleren Reiz-Intervalle in der genannten Arbeit

Über das deutliche Minimum der $N_1$- und $P_2$ Latenzen bei kurzen Intervallen in Abb 4 wird von keinem der zitierten Autoren berichtet Die Vermutung, daß das Ansteigen der Latenzen bei kurzen ISI artifiziell ist und eventuell durch eine Überlagerung der Reizantwort mit dem Off Effekt der Antwort auf den jeweils vorhergehenden Reiz verursacht wird, ist nicht begrundet Nach Messungen von Johannsen et al (1972) sind die Latenzen des Off-Effektes verglichen mit dem On-Effekt kürzer, so daß auch bei 0,2 s Reizintervallen sich nur sehr spate Komponenten ($t_L > 300$ ms) der Off Antwort mit dem $N_1$-$P_2$-Komplex der nächsten On-Antwort überlagern konnen Da die Amplitude des Off-Effektes jedoch bei der verwendeten Reizart nur 10–20% der Amplitude der On-Antwort beträgt (Spreng, 1969), kann der Einfluß einer Überlagerung vernachlässigt werden

Von Beagley & Kellogg (1970) wurden Reizstärke–Erregungskennlinien für Intervalle von 20 s und 1,25 s angegeben, die sehr gut mit den entsprechenden Kennlinien der Abb 5 übereinstimmen, wenn berucksichtigt wird, daß eine Verlängerung des Intervalls von 10 s auf 20 s keinen bedeutenden Amplitudenzuwachs erzielen kann Ein Vergleich mit den von Stange (1971) veroffentlichten Kennlinien für den adaptierten und den nichtadaptierten Zustand ist schwierig, da keine Angaben über die beiden mittleren Reiz Intervalle gemacht werden Auch wird in der genannten Arbeit der logarithmische Maßstab für die Amplituden bevorzugt, während für unsere Meßergebnisse die lineare Darstellung geeigneter erscheint

Die physiologische Grenze der Fähigkeit zur Antwortsteigerung bei Erhohung der Reizstärke ist eine Funktion des Reiz-Intervalls Aus Abb 5 geht hervor, daß bei 1 s Intervallen diese Grenze bei etwa 75 dB ü S erreicht ist Bei

10 s Intervallen liegt sie sicher oberhalb 100 dB ü S Wegen der Unzumutbarkeit für die Versuchspersonen wurden oberhalb 90 dB ü S keine Messungen durchgeführt

Die Ergebnisse wurden im Rahmen der WK Herz-Kreislauf Erkrankungen im Auftrage des Ministeriums fur Gesundheitswesen der DDR erarbeitet Wir danken dem Leiter der Arbeitsgruppe MdT Herrn Oberarzt Dr Michel sowie Herrn Dr Ulrich fur die Unterstutzung

## SUMMARY

The dependence of the $N_1$–$P_2$ amplitudes and latencies of auditory evoked potentials upon the interval between the stimuli (0 2 up to 10 s) was measured at 20, 30, 50, 70, and 90 dB above the audiometer threshold (ISO) Within the investigated range of intervals, the curves (the 20 dB-curve excluded) do not reach their top value Extrapolation of the curves beyond 10 s shows, that at least 90% of the maximum amplitude will be reached at 10 s The percental decrease of the amplitudes at shorter intervals (related to the value at 10 s) will be approximately equal for all intensities When altering the drawing of the measured amplitude values, a curve set of input–output functions (amplitude vs sound level with the duration of intervals as parameter) will be obtained With increasing duration of intervals, the $N_1$- and $P_2$-latencies increase significantly, i e $t_{LP2}$ more than $t_{LN1}$ At short intervals, both latency curve sets run through a minimum Alteration of the sound level causes parallel displacement of the curves only, the dimension of which is a function of the sound level Due to this behaviour, the latency difference of the answers for long and short intervals is approximately independent on the stimulus intensity

## LITERATUR

Beagley, H A & Kellogg S E 1970 Amplitude of the evoked response at high intensities *Sound 4*, 86

Butler R A Spreng M & Keidel W D 1969 Stimulus repetition rate factors which influence the auditory evoked potential in man *Psychophysiology 5*, 665

Davis, H, Mast, T., Yoshie, N & Zerlin, S 1966 The slow response of the human cortex to auditory stimuli recovery process *Electroenceph Clin Neurophysiol 21*, 105

Fruhstorfer, H, Soveri, P & Järvilehto, T 1970 Short-term habituation of the auditory evoked response in man *Electroenceph Clin Neurophysiol 28*, 153

Gjerdingen D B & Tomsic, R 1970 Recovery functions of human cortical potentials evoked by tones, shocks, vibration and flashes *Psychonom Sci 19*, 228

Johannsen, H S, Keidel, W D & Spreng M 1972 Der Einfluß von Intensität und Dauer der Beschallung auf den Off Effekt des akustisch evozierten Potentials. *Arch Ohr Nas Kehlkopfheilk 201*, 208

Karnahl, T & Benning C D 1972 Effect of sedation upon evoked response audiometry amplitude and latency vs sound pressure level *Arch Ohr Nas Kehlkopfheilk 201*, 181

Muller, G & Stange, G 1971 The input–output function of the slow evoked potential in contralateral, ipsilateral and vertex recording *Arch Ohr Nas Kehlkopfheilk 198*, 116

Nelson, D A & Lassman, F M 1968 Effects of intersignal interval on the human auditory evoked response *J Acoust Soc Amer 44*, 1529

Rothman H H, Davis, H & Hay, I S 1970 Slow evoked cortical potentials and temporal features of stimulation *Electroenceph Clin Neurophysiol 29*, 225

Ruhm, H B 1971 Lateral specificity of acoustically evoked EEG responses I Non verbal, non meaningful stimuli *J Aud Res 11*, 1

Spreng M 1969 Problems in objective cerebral audiometry using short sound stimulation *Int Audiol 8* 424

Stange, G 1971 The relation between functional parameters of subjective and objective audiometry *Arch Ohr Nas Kehlkopfheilk 198*, 85

Webster, W R 1972 The effects of repetitive stimulation on auditory evoked potentials *Electroenceph Clin Neurophysiol 33*, 159

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# LABYRINTHINE MORPHOLOGY AND TEMPERATURE IN CRYOSURGERY (GUINEA PIG)

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(Received July 27 1973)

*Abstract* The effects of cryosurgery on the guinea pig labyrinth as visualized by light and electron microscopy are presented The material studied was taken from animals in which the intralabyrinthine temperatures were known (Morrison & Lundquist 1973) Between +18 and +19°C changes first appear in the mitochondria located in the apical part of hair cells close to the endolymph At cooler temperatures intracytoplasmic vacuoles appear clumping of nuclear chromatin can be seen and as the changes progress they involve the supporting cells secretory areas and connective tissue as well At even colder levels (+4° +10°C) the epithelium becomes fragmented and lifts away from the connective tissue However this debris is cleared away within 4 days and the crista is healed by a layer of flattened supporting cells

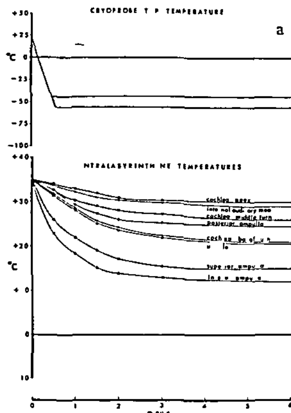
Several surgical procedures have been developed in an attempt to relieve patients with Meniere s disease of their vertigo and tinnitus without causing further deterioration of hearing A recent method is the cryosurgical alteration of vestibular function achieved by placing a cryosurgical probe on the lateral semicircular canal (Cutt et al , 1965, Wolfson & Cutt, 1971, Wolfson, 1973, Tabor et al , 1972) Little is known about what actually takes place within the labyrinth during cooling In a recent publication Morrison & Lundquist (1973) presented the temperature changes that can be achieved in various parts of the guinea pig labyrinth when the lateral semicircular canal ampulla is cooled by a cryosurgical probe, and the advance of the cooling front was shown for different probe tip temperature levels The intralabyrinthine temperature recordings were correlated with morphological changes as seen by light microscopy This investigation presents, in greater detail, the histological changes caused by a cryosurgical intervention in the labyrinth The influence of survival time and the relationship to intralabyrinthine temperatures will be discussed as well

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[2] Supported by grants no B73 17X 3915-01 and B74-17X 3915-02 from the Swedish Medical Research Council and by funds from the Karolinska Institute

## MATERIALS AND METHODS

Materials for morphologic study were taken from 18 young guinea pigs in which the bone over the left lateral semicircular canal ampulla was exposed to a cryosurgical probe at two temperature levels, −50°C and −75°C, for various periods of time The animals were further subdivided into groups having different survival times after the freezing procedure, so that specimens were taken either immediately after freezing utilizing an intravital labyrinthine perfusion of osmium, or else the animal was allowed to live for 2 hours, 1 day, or 4 days after cryosurgery After removal of the temporal bones the intralabyrinthine structures were fixed in 1% cold osmium tetroxide, dissected, dehydrated, embedded in Epon, and cut on an LKB Ultrotome



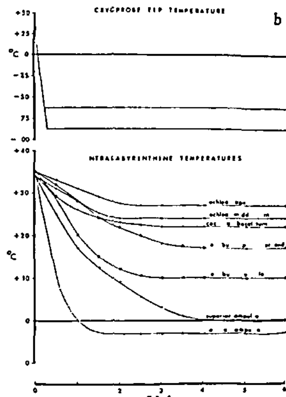




*Fig 1* Temperature changes in the guinea pig labyrinth during the application of a cryoprobe on the lateral ampulla maintained at −50°C (*a*) or −75°C (*b*) (Taken from Morrison & Lundquist 1973)

For examination by light microscopy 1 μm thick sections were used, stained with toluidine blue Thin sections mounted on copper grids were stained with uranyl acetate and lead citrate and examined with a Siemens Elmiskop I electron microscope

The cristae from the lateral and superior semicircular canal ampullae as well as the macula of the utricle were examined in each case Cochlear preparations, as well as preparations of the saccule and posterior ampulla, were made from several of the more severely cooled specimens

## RESULTS

The morphologic changes caused by cryosurgery on the vestibular labyrinth will be presented in two main sections Firstly, we will examine the various changes seen immediately after the cooling procedure and secondly, the effects of survival time after cryosurgery will be described

### *Immediate morphological effects of cooling*

When the guinea pig labyrinth is cooled by a cryosurgical probe a very wide range of morphological changes may be produced, depending on several factors, the most important being the degree of cooling With the application of a −50 to −75°C cryoprobe on the lateral semicircular canal ampulla, intralabyrinthine temperatures from −3°C to +30°C are obtained (Fig 1) The temperatures described are actual intralabyrinthine recordings.

### *Minimal changes*

When the epithelial temperature is lowered to +18 or +19°C the morphological changes are very slight and can only be seen with the



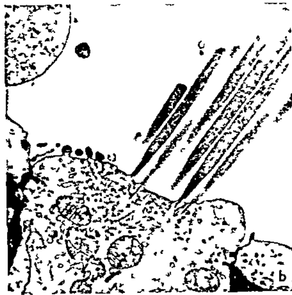
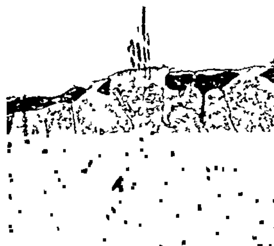




*Fig 2* Light micrograph (*a*) and electron micrograph (*b*) of a lateral ampullary crista that had been subjected to very minimal cooling By light microscopy it is normal appearing however electron microscopy reveals early mitochondrial degeneration (*a* Toluidine blue ×200 *b* ×10 000)

electron microscope This is demonstrated by Fig 2 in which the light micrograph of an ampullary crista is normal appearing, but electron microscopy reveals some destruction of the internal cristae of the mitochondria, especially in the apical region of the hair cells close to the endolymph At slightly lower temperatures (+10 to +15°C) vacuoles begin to appear in the cytoplasm, initially in the subapical and supranuclear regions but with still more cooling they are seen throughout the hair cell cytoplasm (Fig 3)



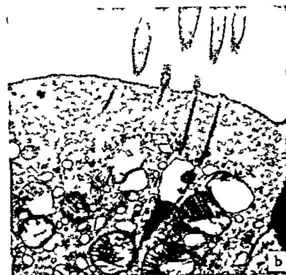



*Fig 3* Guinea pig crista epithelium exposed to an intralabyrinthine temperature of about +10–+15°C There is widespread damage to mitochondria and cytoplasmic vacuole formation Fig 3 *b* is a higher magnification of the apex of the type I hair cell shown in (*a*) (*a* ×2 500 *b* ×13 000)



*Fig. 4.* Ballooning of surface membranes and extrusion of cytoplasm in type I hair cells mildly affected by cryosurgery. The kinocilia are distorted. Note the mitochondrial changes as well. (×22 500.)

Some ballooning of the hair cell surface membrane and cytoplasm into the endolymph, alongside the cuticular plate, is frequently seen in normal specimens and is attributed to fixation artifact. However, in the mildly affected cryosurgical specimen this is more widely spread, and an increase in cytoplasmic extrusions can be seen (Fig. 4).

Slightly beyond the stage of these most subtle changes some clumping of nuclear chromatin becomes visible, and at this stage of degeneration changes are usually visible in the light microscope.

*Moderate changes*

These occur at a temperature of approximately +4 to +10°C. As mentioned above, the more extensive intracellular vacuolization together



*Fig 5* Early cryosurgical changes as seen on light microscopy There is noticeable edema between the epithelium and connective tissue in the transitional zone, and very early changes can just be detected in the sensory areas (Toluidine blue, × 235 )







Fig 6 (*a*) Large vacuoles and empty mitochondria are seen in the hair cell cytoplasm to the right Further left is the nerve chalice containing distorted mitochondria as well The supporting cell (top left) is normal appearing ( × 22 000 ) (*b*) Well developed myelin figures in a nerve chalice ( × 38 000 )



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*Fig 8* Light micrographs of two lateral cristae each exposed to a −75°C cryoprobe for 3 minutes (Intra labyrinthine temperature about −3°C) Note the epithelial fragmentation nuclear pykrosis and cellular debris floating into the endolymphatic fluid (Toluidine blue ×200)

specimens taken from regions of the vestibular system where the degree of cooling was greatest Again however, the damage is found to be more extensive in the hair cells than in other elements

The more severely damaged mitochondria are usually seen as distended, almost empty envelopes, but myelin figures may also be present, and are considered to be remnants of destroyed mitochondria (Fig 6) Mitochondrial changes in the nerve endings are less pronounced than those seen in the hair cells

Prior to the most severe changes with break-up of the epithelium, the intracellular vacuoles coalesce and become larger, and quite large vacuoles are now found in supporting cells as well (Fig 7)

*Severe changes*

Typical severe changes in the lateral semicircular canal ampulla as seen by light microscopy are shown in Fig 8 The sensory epithelium is generally disorganized and in many areas it has lifted away from the underlying connective tissue However, even in the midst of areas of marked destruction some continuity of the reticular lamina can be seen Many of the nuclei are pyknotic Cellular debris is found floating in the endolymphatic fluid The epithelial disruption is consistently more pronounced on the canal side of the ampullary crista, which is the one closest to the site of cryoprobe application

The central core of the crista is edematous and the blood vessels are congested with red blood cells The dark cells of the secretory region appear, on light microscopy, to be irregular but have survived the cooling much more than the sensory areas above them

The electron microscopic appearance of the epithelial disruption can be seen in Fig 9 All cells are fragmented and most of the nuclei are pyknotic The apical region of a destroyed hair cell can be seen in which the general relationship between the reticular lamina and the cuticular plate is maintained, however the zones of contact between the cells, with the tight junctions, are lost The arrangement between the sensory hairs and the cuticle is intact as are the hair rootlets within it, however

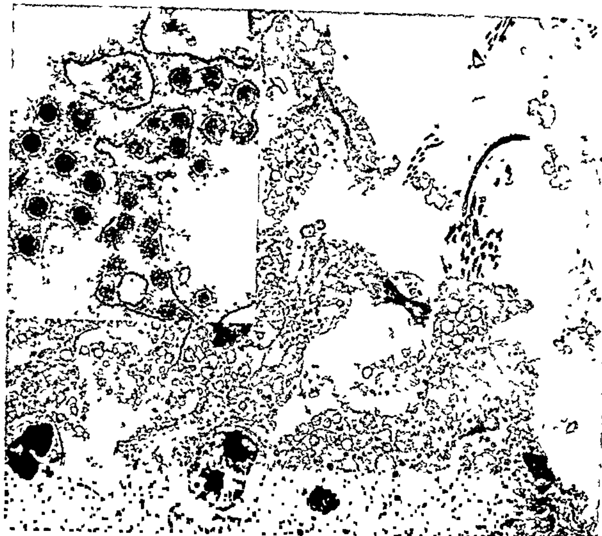


Fig 9 Electron micrographic survey of epithelial disruption similar to that in figure 8 The top of a hair cell can be seen in which the relationship to the reticular lamina is maintained The inset shows a cross section of a sensory hair bundle in this region Note how the central cores of the stereocilia have retracted away from the surrounding limiting membrane (× 5 000 Inset × 43 000)

the structure of the hairs is altered This is shown in the inset where the central cores of the stereocilia seem to have become shrunken and separated from the surrounding limiting membrane In one region the cores of several stereocilia appear to be in the process of being surrounded by a common membrane The membrane around the kinocilium is also distended, but the internal fibrils are still normal in appearance

The basement membrane is usually found to be intact, with some fragments of supporting cell cytoplasm and membrane adherent to it However, in other areas all epithelial structures are completely gone (Fig 10) Severe cryosurgical changes can also be seen in the subepithelial regions where the vessels are congested, although the endothelial lining is intact In the myelinated nerve fibers there is degeneration of mitochondria, shrinking of axoplasm, and disruption of the myelin sheath

Fig 11 shows the secretory cell region from the side of an ampulla in which the sensory cells have been completely destroyed The dark cells are surprisingly normal appearing when compared with the surrounding chaos As can

be seen the pigment cells below the dark cells are completely destroyed with free pigment granules dispersed in the connective tissue The mitochondria and cytoplasmic processes are normal appearing and active pinocytosis can be seen at the endolymphatic surface The nucleus is as in the normal state somewhat irregular (Kimura et al 1964)

*Effect of survival time after cryosurgery*

Very little difference is discernible on light microscopy between a specimen taken immediately after cryosurgery and one taken two hours later (Figs 12 *a* and 8 *a*) The amount of epithelial fragmentation and vacuole formation is very similar in both instances These findings are confirmed on electron microscopy

In specimens taken 1 day after the cryosurgical exposure (Fig 12 *b*) it can be seen that the epithelial debris is beginning to "float away from the connective tissue of the crista and the remaining viable part of the sensory epithelium has tapered down to even out the cristal surface Electron micrographs of this region show that it is the supporting cells that are flattening down to give this appearance

After 4 days a thin cell layer has again covered the connective tissue (Fig 12 *c*) in which the vascular and neural elements have been replaced by a fibrous substance An electron micrograph of this region (Fig 3) shows the healing cell layer to be composed of greatly flattened and elongated supporting cells A thickened basement membrane lies between these cells and the fibrinous deposit below



*Fig 11* Electron micrograph of the dark cell area from a crista in which the sensory epithelium had been destroyed The pigment cells are disrupted but the dark cells are intact. The mitochondria and cytoplasmic processes are normal appearing (× 1 400)



*Fig 10* The sensory epithelium has lifted away from this portion of ampullary crista leaving an exposed basement membrane Note the destruction of mitochondria in nerve axons and the disorganization of myelin sheaths in the connective tissue (× 1 300)

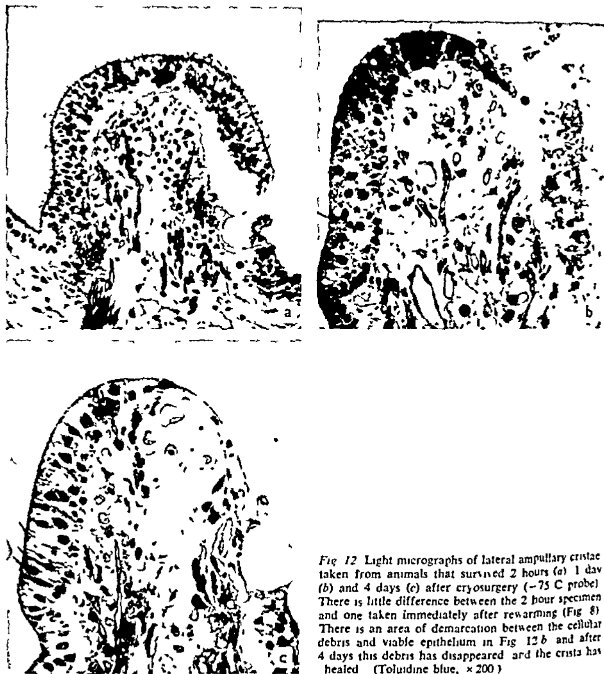



*Fig 12* Light micrographs of lateral ampullary cristae taken from animals that survived 2 hours (*a*) 1 day (*b*) and 4 days (*c*) after cryosurgery (−75 C probe) There is little difference between the 2 hour specimen and one taken immediately after rewarming (Fig 8) There is an area of demarcation between the cellular debris and viable epithelium in Fig 12 *b* and after 4 days this debris has disappeared and the crista has healed (Toluidine blue, ×200)

Some giant hairs can be seen in a few of the remaining hair cells on the side of the crista opposite the major damage in this same specimen (Fig 14)

*Utricular changes*

At vestibular temperatures of +10°C the utricle still appears to be morphologically intact Even in the preparations where the lateral canal ampullary crista was completely destroyed only very minimal changes were ever visible in the utricular epithelium by light microscopy When this specimen was examined by electron microscopy moderate mitochondrial changes plus a few cytoplasmic vacuoles could be seen However in some cases where there was relatively severe damage to the lateral crista (Fig 7) the utricle was completely nor



*Fig 13* Four days following the cryosurgery the basement membrane is seen to be covered by a layer of flattened supporting cells These are felt to migrate in from the periphery of the lesion. The basement membrane is slightly thickened and below it there is an increased amount of fibrinous material (× 6 900)

mal on both light and electron microscopy (Fig 15) The possible reasons for this remarkable utricular resistance will be discussed in the next section

## DISCUSSION

The various morphological effects of cryosurgery on the guinea pig vestibular labyrinth have been presented through the range of minimal changes to severe epithelial disruption Our findings suggest that the main disruption occurs immediately after the cryosurgical exposure As demonstrated in a previous paper (Morrison & Lundquist, 1973) many of these epithelial changes can be produced without actually cooling the epithelium to the freezing point, in fact moderately severe intracellular damage can be done to hair cells when they are cooled to only +18°C or +19°C As has been shown, the earliest changes include destruction of the internal cristae of the mitochondria and vacuolization of the hair cell cytoplasm These changes then progress to pyknosis of nuclei and general epithelial fragmentation Following this degree of destruction the disorganized epithelium becomes separated from the underlying connective tissue, and within 4 days the cellular debris has been cleared away from the ampullary region

Within the sensory epithelium the hair cells and nerve endings are most susceptible to cryosurgical damage, and the supporting cells less so The dark cells of the secretory region also seem less susceptible to damage than other cells around them, such as pigment cells or the hair cells

The changes present are similar to those found by Wersall et al (1965) in a study of postmortal degeneration However, in that investigation it was stressed that the mitochondrial disturbances first appeared in the nerve



*Fig 14* Giant hair found on the less affected side of the specimen shown in Fig 12 *c*, taken 4 days after cryosurgery (× 16 400)



*Fig 15* Normal type I and type II hair cells in a utricle that had been cooled to +20 C (× 2 500)

endings because of their higher metabolic rate, in contrast to cryosurgical damage in which the changes are first seen in the apical cytoplasm In both cases the degeneration is probably a result of a metabolic block, but in the cryosurgical specimens the nerve endings are shielded from the cold

That the dark secretory cells are comparatively undisturbed could be explained by their position close to the protective vascular network of the bony ampullary wall

The severe cryosurgical trauma that leads to epithelial disruption also affects the basement membrane to some extent, and it is interesting to find that some pieces of cytoplasmic membrane are attached to the basement membrane residues This might lend support to the theory that the basement membrane is produced by the epithelial cells themselves, rather than the connective tissue (Pierce, 1965, Eriksson & Frithiof, 1973)

After 4 days however, the denuded areas are again covered by an epithelial lining consisting of supporting cells resting on a once again intact basement membrane In the supporting cells of other animals, particularly the squirrel monkey, striated inclusions bodies can be found close to the centrioles, which might be interpreted as sign of capability of cell division, as has been suggested by Spoendlin (1966) In the present study no evidence of cellular division was found, and the manner in which the supporting cells are stretched out over the basement membrane suggests that they have not been newly formed An active migration of these supporting cells from the wound edges is thought likely to occur Further investigation of this healing process is underway utilizing scanning electron microscopic techniques

As mentioned above the utricle is remarkably resistant to cryosurgical destruction There are several possible reasons for this Firstly,

the bone against which the utricle lies is on the opposite side of the vestibule from the lateral and superior ampullae so that it would be less affected by conduction of the cold through bone Secondly, the cooling front arriving at the utricular region must cross the inner ear fluids, and as a result may be dissipated in various directions Also, the epithelial surface of the utricle is protected to some extent by the otolithic membrane which may act as a blanket, isolating the epithelium from the temperature gradient It has been shown by Stahle (personal communication) that the blood supply below the utricular epithelium is quite extensive, more so than in the connective tissue of the ampullary cristae This would give the circulatory system a better chance to keep the utricle warm during the cryosurgical procedure

From the present investigation we would suggest that the main effects of cooling on the vestibular epithelium are the result of a metabolic block, and not caused by actual freezing or ice crystal formation The tissue can of course, be frozen if lower temperatures are used and these effects have been shown by Lundquist et al (1972)

## ACKNOWLEDGMENT

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## ZUSAMMENFASSUNG

Die Wirkung der Kryochirurgie auf das Labyrinth des Meerschweinchens wird nach licht und elektronenmikroskopischer Untersuchung dargestellt Das untersuchte Material stammte von Tieren bei welchen die intralabyrinthären Temperaturen bekannt waren (Morrison & Lundquist 1973 im Druck) Bei Temperaturen zwischen +18 und +19°C treten Veranderungen zuerst an den Mitochondrien im apikalen Teil der Haarzellen nahe der Endolymphe auf Bei niedrigeren Temperaturen konnen intrazytoplasmatische Vakuolen und Verklumpungen von Kernchromatin beobachtet werden bei fortschreitenden Veranderungen werden Stutzzellen sekretorische Regionen und auch das Bindegewebe betroffen. Bei noch niedrigeren Temperaturen (+4° bis +10°C) kommt es zur Fragmentation und Abhebung des Epithels vom Bindegewebe Diese Zelltrummer werden jedoch innerhalb von 4 Tagen beseitigt, und die Crista verheilt mittels einer Schicht von abgeflachten Stutzzellen

## REFERENCES

*Cutt R A Wolfson R J* Ishiyama E Rothwolf F & Myers D 1965 Preliminary results with experimental cryosurgery of the labyrinth *Arch Otolaryng* (Chic) *82* 147

Eriksson, G & Frithiof L 1973 The basement membrane in normal skin and split skin autografts in man *Scand J Plast Reconstr Surg* in press

Kimura R Lundquist P-G & Wersall J 1964 Secretory epithelial linings in the ampullae of the guinea pig labyrinth *Acta Otolaryng* (Stockh) *57* 517

Lundquist P-G Igarashi M Wersall J Alford B R & Wright W K 1972 Effects of cryosurgery on vestibular sensory epithelia in the guinea pig An electron microscopic investigation *Arch Otolaryng* (Chic) *95* 530

Morrison M D & Lundquist P-G 1973 Intralabyrinthine temperature changes during cryosurgery in the guinea pig In press

Pierce G B Jr 1965 Basement membranes VI Synthesis by epithelial tumors of the mouse *Cancer Res 25* 656

Spoendlin H 1966 Ultrastructure of the vestibular sense organ In *The vestibular system and its diseases* (ed R J Wolfson) pp 39–68 Univ of Pennsylvania Press Philadelphia

Stahle J 1973 Personal communication

Tabor J R Best L & Donohoe R W 1972 Results in labyrinthine cryosurgery monitored by evoked response *Laryngoscope 82* 1431

Wersall J Kimura R & Lundquist P-G 1965 Early postmortem changes in the organ of Corti (guinea pig). *Z Zellforsch 65* 220

Wolfson R J 1973 Labyrinthine surgery for Meniere s disease In *Otolaryngologic Clinics of North America* Vol 6 No 1 pp 131 138

Wolfson R J & Cutt R A 1971 Long term results with cryosurgery for Meniere s disease *Arch Otolaryng* (Chic) *93* 483

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*Fig 14* Giant hair found on the less affected side of the specimen shown in Fig 12c taken 4 days after cryosurgery (× 16400)

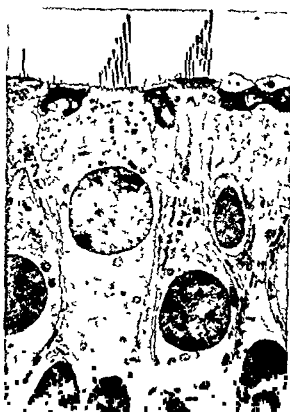


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## REFERENCES

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Stahle J 1973 Personal communication

Tabor J R Best L & Donohoe R W 1972 Results in labyrinthine cryosurgery monitored by evoked response *Laryngoscope 82* 1431

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# ERRONEOUS PERCEPTION OF VERTICAL MOTION BY HUMANS SEATED IN THE UPRIGHT POSITION

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*Abstract* The reliability of subjective response to vertical linear accelerations in the absence of vision has been examined in human subjects seated in the upright position Helicopters and a vertical movement simulator were used to generate sinusoidal vertical movement of period 2–10 sec and acceleration amplitude 0 2–0 4 *g* Subjective response was recorded as a continually variable assessment of vertical position Without prior knowledge of the movement pattern, subjects were aware of movement but registered its form with a performance little better than chance Light to moderate simulated "turbulence" did not significantly alter performance Detailed consideration of end-organ orientation in the skull suggests the possibility that relative paucity of vestibular afferent information about vertical movement may account at least in part for the poor tracking performance observed in these experiments

The quoted thresholds of human sensation to linear acceleration appears to be in the order of 0 005 to 0 01 *g* (Jongkees & Groen, 1946, Walsh, 1961, 1962 and 1964, Meiry, 1965, Young & Meiry, 1968) However, pilot experience in aircraft, especially helicopters and VTOL aircraft (Malcolm, 1971) suggests that the perception of vertical motion (i e parallel to the gravity vector) in an erect, seated, position is in practice considerably less effective than would be expected from this order of sensitivity Possibly this apparent discrepancy might arise from the fact that none of the investigations cited above used specifically *vertical* accelerative motion with the subject seated in an *upright* position

*Horizontal* accelerations, whether parallel or transverse to the body s long axis, introduce changes in direction of the resultant linear acceleration vector due to summation of horizontal and gravitational accelerations, and this has been shown of itself to generate significant physiological response (Guedry, 1965, Benson & Bodin, 1966, Niven et al, 1966, Benson et al, 1970) Walsh (1964) studied response to vertical motion but the long axis of his subjects was horizontal As discussed later, the anatomical arrangement of the vestibular organs in the skull could well lead to discrepancy between results obtained in this way and similar ones obtained with a subject seated erect The following experiments were therefore designed to investigate the ability of human subjects to track purely vertical accelerative motion when seated erect

## METHODS

The experiments were conducted in three phases, of which the first and second employed helicopters in real flight and the third used the NASA Height Control Apparatus (HCA) with the generous co-operation of NASA Ames Research Center

This research was supported by Canadian Defence Research Board Grant Numbers 9910-37 and 9310-92

*Phase one (Preliminary Experiment)*

Ten male military personnel, five of whom were aircrew, were seated in the back of a large transport helicopter They wore blind folds and were asked to signal the direction in which they felt they were moving by means of pre arranged hand signals Using manual control, the pilot of the helicopter tried to approximate vertical sinusoidal oscillations having peak to peak accelerations of 0 4 to 0 6 g, that is, well in excess of the threshold values referred to above He was asked to perform 20 cycles each of oscillations having periods of 2, 4, 6, 8 and 10 sec/cycle An onboard observer maintained visual contact with the ground and surrounding nearby hangars, and by means of the same kind of hand signals as the subjects indicated the actual motion of the helicopter The entire procedure was filmed on 16 mm cine film for later analysis

The subjects had been told that they would experience random motions representing different types of turbulence The films revealed that they quickly got out of phase with the true motion of the machine, some even reporting that they were moving in precisely the opposite direction to that of the real helicopter motion!

These surprising preliminary findings obviously warranted further investigation with better methods of applying the stimulus movement and of recording the human response

*Phase II (Computer Controlled Helicopter)*

The National Aeronautical Establishment of the National Research Council of Canada provided the services of a helicopter which could be completely controlled by an onboard analogue computer

*Subjects* Useful data were obtained from 6 volunteers who were employees of the National Aeronautical Establishment Ages ranged from 21 to 40 years, and 3 of the subjects were current pilots, 2 were women

*Stimuli* The computer was programmed to fly the helicopter through 20 cycles in each of five runs having successive periods of 2, 4, 6, 8 and 10 seconds Each subject was exposed to all five runs The accelerations of the helicopter, as measured by its onboard accelerometers, closely approximated the intended sinusoidal pattern The peak-to-peak acceleration in all the runs was 0 4 *g* The nature of the control program was such that the sinusoidal oscillations were superimposed on a steady rate of ascent This latter was not detectable by the subjects, and no evidence could be found that it influenced the data

*Data acquisition* As in Phase I, the subjects were told that the motions of the helicopter would be random vertical movements attempting to approximate different types of turbulence The helicopter accommodated one subject at a time along with the pilot who controlled the experiment The subject was equipped with a signalling device strapped to his right thigh as illustrated in Fig 1 This device consisted of a box from which protruded two handles, one fixed (to act as a reference) and the other free to move back and forth through 90°, corresponding to a handle travel of some 5 to 6 inches By means of this lever, the subjects were asked to indicate their perceived altitude They were to move the handle forward if they felt they were high with respect to the starting point, and back towards their stomachs if they felt they were low The lever was attached to a potentiometer battery combination which produced a bipolar analogue voltage proportional to the displacement of the handle This signal was then fed to the helicopter's flight recording apparatus, along with the outputs from the onboard accelerometers Fig 1 shows one of the subjects operating the signalling device while seated in the helicopter

*Special conditions* A considerable amount of noise was generated by the helicopter's motor and rotors, as well as by aerodynamic noise

from the cockpit walls To prevent a subject from gaining possible cues from this noise, he wore a pair of highly attenuating earphones (Sharpe HA 10, Mk 2), which were driven by two loud audio tones One tone was fixed in frequency at 1 kHz while the other tone varied in pitch according to the position of the signalling handle When the handle was in the center position, the tones were adjusted so that they were identical, and when the subject signalled he was high, one of the tones would increase in frequency accordingly, and vice versa The apparatus used to generate these tones was contained in a second box strapped to the subject's other thigh The subjects kept their eyes tightly closed at all times during the runs

*Phase III (NASA Height Control Apparatus)*

As will be seen below, the results of the Phase I and II experiments were quite unexpected Possibly the subjects were being confused by disturbances in the stimulus caused by turbulence In order to investigate this aspect of the problem in greater detail, as well as to expand the data field of the helicopter experiments, another experiment was performed on the Height Control Apparatus (HCA) at NASA s Ames Research Center in California This device is an enclosed aircraft cockpit, without windows mounted on runners and wheels such that it can move up and down a vertical track which is fixed to the side of a large building The HCA is controlled by a computer, and is capable of moving smoothly through a vertical displacement of 80 ft with a 10 ft safety margin at each end

*Subjects* The subjects used in this phase were two women and eight men, ranging in age from 27 to 49 years none of whom was a pilot

*Stimuli* The subjects were again exposed to 20 cycles each of oscillations having successive periods of 2, 4, 6, 8 and 10 sec/cycle



*Fig 1* Subject seated in helicopter operating handles of signal box Two loud tones in the earphones masked noise cues associated with the motion

In this series however, the peak to-peak amplitude of the accelerations was 0 2 *g* This somewhat lower value was necessitated by the limited travel available on the simulator as compared with the helicopter However, it is at least an order of magnitude higher than the threshold values quoted in the introduction

Two different levels of turbulence were simulated on the HCA A sample of accelerometer records from these as well as the control runs is shown in Fig 2 The control stimuli (top trace) provided the smoothest movement available on this device Light turbulence was simulated by superimposing on to the driving function of the control computer a noise signal whose power spectrum had a gaussian distribution centered at 8 Hz The resultant accelerometer record is shown as

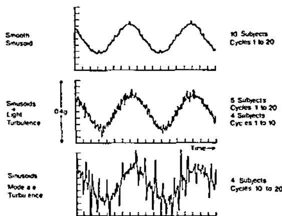



*Fig 2* Samples of accelerometer records from the Height Control Apparatus at NASA Ames Research Center

the center tracing of Fig 2 A moderate degree of turbulence was simulated by introducing random electrical pulses of short duration which would cause the cab of the HCA to lurch up or down as shown in the lowest trace of Fig 2 It is worth emphasizing that the acceleration records in Fig 2 are displayed at relatively high gain In practice, the perturbations seen on the top trace are barely perceptible, whilst the bottom trace is indeed equivalent to moderate turbulence

All subjects were exposed to the smooth sinusoids (control runs) as shown in the top trace of Fig 2 They were then divided into two groups One group of five repeated the control experiment to which had been added the light turbulence, and the second group of four (one man being unavailable) was subjected to the moderate turbulence during the last half of each run

*Data acquisition* The subjects were asked to signal their perceived height with respect to the starting point of each run with the movable lever system described above This time, however, the box was mounted on a vertical column fixed to the floor of the cab and positioned between their knees This allowed the lever to be moved up and down instead of fore and aft, an arrangement felt to be more closely allied to the real vertical motion The subjects were instructed to rest their right forearm on their right thigh, in order to minimize the introduction of artifacts due to inertia of the arm The subject's responses were fed by cable to a pen recorder, along with signals proportional to the acceleration, height and velocity of the cab

*Special conditions* The earphones and tone generators were used again in order to minimize extraneous auditory cues The subjects seated upright in an aircraft seat with shoulder and lap restraint They were instructed to keep their eyes closed throughout the runs

Unfortunately, a condition for using the HCA required the experimenter to show the subjects the exact nature of the stimuli they would be exposed to It was later realized that the subjects' knowledge of the periodic nature of the motion may have significantly altered the manner in which they responded to the stimuli, a matter which will be discussed at greater length below

## RESULTS

*Phase II*

A sample of the original data obtained from one subject during the computer-controlled helicopter phase of the experiment is shown in Fig 3 The upper trace (1) is a record from the accelerometer which was sensitive to the vertical acceleration of the helicopter

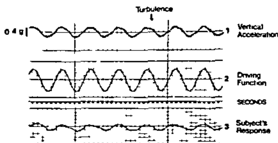



*Fig 3* Sample of raw data obtained from a subject in the computer-controlled helicopter The distortion in the sinusoidal pattern labelled "turbulence" was unintentional Note how the subject "tracks" this perturbation

Trace (2) is a record of the sine wave voltage used as the forcing function to drive the helicopter's flight control computer Trace (3) is a record of the subject's tracking response In this example, the subject was tracking the real movement rather well, but as will be seen below, this was by no means typical Note how, when the acceleration record was distorted by turbulence, the subject quickly signified that something had happened, indicating that he was concentrating on tracking the motion of the helicopter This degree of concentration was typical of all subjects Additional traces of rates of yaw, pitch and roll, not shown here, confirmed that computer stabilisation in these three degrees of freedom effectively eliminated any cross-coupled aircraft motion

These data were reduced by measuring the delay of the subject's peak response with respect to the peak of the stimulus From this, was calculated the phase angle of the peak

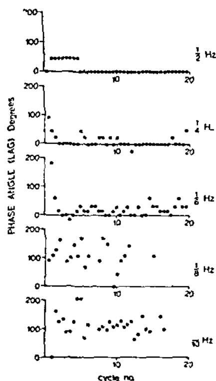



*Fig 4* Phase angle (lag) of the subject's response as compared with his actual position in space There is one dot for every half cycle of response

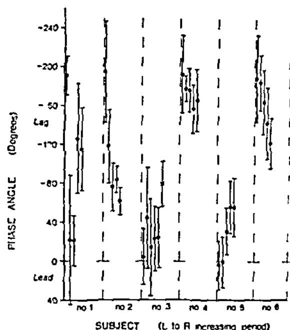



*Fig 5* Average phase angle of the subjects response as compared with their actual position for all subjects and all runs Each dot represents the mean phase angle averaged over the twenty cycles of each run Range bars indicate one standard deviation For each subject displayed along the horizontal axis the period of the motion is shown increasing from left to right from 2 sec to 10 sec

responses with respect to the peak stimuli for each cycle of each run Fig 4 shows graphs of the phase angles of a subject's responses for each of the five periods to which he was exposed In this and all other graphs plotting phase, a negative sign on the ordinate indicates phase lag of response relative to stimulus In this subject's responses, there was a tendency for increasing phase lag to be associated with decreasing frequency However, the change with frequency was not consistently progressive Moreover, no individual subject was representative of the whole group

Fig 5 gives the average phase angle for an entire run (20 cycles) as a function of the period of the oscillation for each of the six subjects tested The data for each subject are separated into six vertical columns by the dashed lines Within each column the mean phase angle and its S D are plotted from left to right in order of increasing period

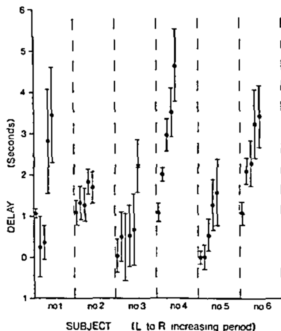



*Fig 6* Average delay of each subject's response for each run As in Fig 5, the dots represent the means over twenty cycles, while the bars indicate one standard deviation The data are displayed in the order of increasing period of oscillation along the horizontal axis for each subject

On visual inspection of Fig 5, it can be seen that there was apparently little or no consistent change of phase with frequency from one subject to the next and this is confirmed by the absence of statistically significant trends between subjects However, in the figure it can be seen that there was a tendency for the results from subjects number 3, 4, 5 and 6 to cluster round the phase angles 0 or 180° This cannot be said of the results from subjects number 1 and 2 The implications of these two features will be discussed later

The points depicted by an X in this and the following figure (6) are the results of asking one subject to repeat the run having a period of 10 sec while keeping the eyes open The sky was quite clear, with unlimited visibility The average height of the helicopter was 1 500 ft above an open field, yet this subject's mean phase error during this run was almost twice as great as that obtained from the same stimulus with eyes closed only minutes earlier It should be noted that during this time, the helicopter underwent changes in altitude of the order of 200 ft!

Fig 6 is derived from the same data as was the previous figure and shows the average delay of the peak response relative to peak stimulus as a function of the period of the motion Once again, each dot corresponds to one subject's results averaged over the 20 cycles of a run at a given period

Upon inspection, it is obvious that a consistent pattern of delay cannot explain the overall results On the one hand, subject number 2 manifested a relatively constant delay in responding to all stimuli, whereas subject number one showed a widely varying delay which cannot even be attributed to a systematic change in phase angle The pattern of delays in the responses from subject number 4 conforms with those responses being consistently about 180° out of phase with the stimulus

*Phase III*

Data from the phase 3 experiments conducted on the Ames Research Center Height Control Apparatus (HCA) were reduced in the same manner as those from the helicopter

Fig 7 shows the phase angle of response as a function of the period of the oscillation for all the runs on the HCA Each point represents the average response of a subject to 10 cycles at a given period of oscillation The open circles are derived from the smooth runs, the closed circles from the light turbulence runs and the triangles from the moderately turbulent runs As can be seen, no significant effect on the subject's responses can be attributed to the turbulence Also of interest is the relatively large proportion of subjects who anticipated the motion of the cab, as signified by the responses which lead the stimulus.

The data offer a means of assessing the reliability of one's perception of motion un-

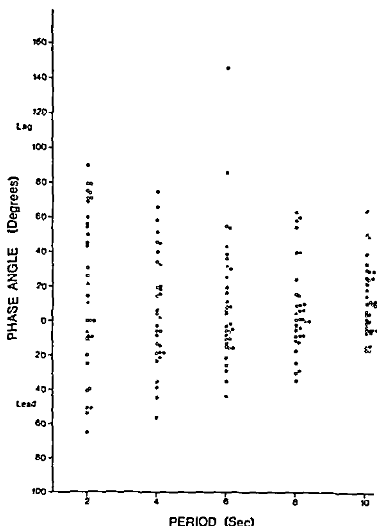



*Fig 7* Average phase angle of subjects' responses compared with position of Height Control Apparatus as a function of the period of the oscillation o data from the smooth runs •, light turbulence runs, Δ, moderate turbulence runs Each point represents the mean of ten cycles

der the conditions of this experiment In the computer-driven helicopter, 586 subject-cycles were completed, and 281 (48%) were out of phase by more than 90 degrees If, during the run, a subject could not make up his mind which way he was moving, he generated a 'blank" response by centering the handle of the signal box and holding it steady Of the responses from the helicopter, 27 (4 6%) were "blank"

In the HCA, 1 943 subject-cycles were completed Of these, 153 (7 9%) were out of phase by 90° or more, 466 (24%) were out by 45° or more, while 301 (15 5%) were "blank" These "blank" responses were predominantly the result of two subjects who became "lost" for the majority of the experiment If these two had reacted with the same assurance as the other subjects on the HCA, the proportion of "blanks" would be similar to that found in the data from the helicopter

## DISCUSSION

An important outcome of these experiments is that, although subjects usually made well-defined oscillatory tracking responses and hence presumably thought they knew what was happening, in practice those responses were likely to be quite wrong It is difficult to decide whether the errors were due to a low signal-to-noise ratio from the senses involved, or to a specific liability to make an incorrect assessment of the direction of move-

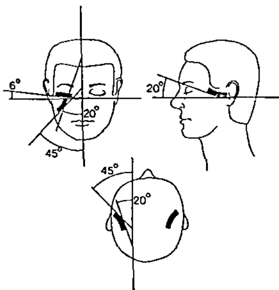



*Fig 8* Planes occupied by the gravity receptor organs Free after, Quix (1923) Miller (1962) and Jongkees (1967)

ment Thus in Fig 5, the widely scattered phase data from subjects 1 and 2 suggests the problem was one of resolving signal from noise, whilst the results from subjects 3, 4, 5 and 6 suggest it may have been largely one of determining the correct direction of movement Both these possibilities could account for the occasional "blank" responses representing frank admissions by the subjects that they were confused

These findings are in accord with those of Correia et al (1968), who noted relative insensitivity of the oculogravic illusion (Graybiel, 1952) to linear acceleration along the body's long axis, especially when the subject's head was tilted forwards 30° as was the case in our experiments On the other hand our results from vertical movement stimuli contrast markedly with corresponding results obtained by Meiry (1965) and Young & Meiry (1968) from subjects exposed to sinusoidal linear accelerations in a horizontal plane The responses of their subjects were sufficiently consistent to define characteristic changes in phase of response with frequency of sinusoidal stimulation, from which a stimulus response transfer function was deduced

It should be emphasised that in all these experiments the subject's whole body was exposed to the varying force field which constituted the stimuli Hence it is quite possible that different patterns of response could be accounted for by subjects responding to

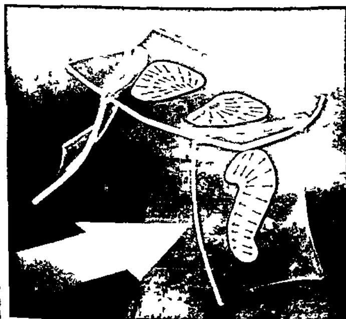


*Fig 9* Plastic model showing the spatial relationship of the gravity receptor organs Large arrow points in the forward direction and lies in the horizontal plane defined by the axes in Fig 8 Small arrows seen on the model indicate presumed direction of maximum excitability of sensory cells in the organs (see text)

different sets of sensory inputs, ranging from the special acceleration sensitive organs of the vestibular system to superficial and deep sensations throughout the body

However, this possibility is rather difficult to reconcile with Walsh's observation (1961) that the sensation threshold of Labyrinthine Defective (L-D) subjects to sinusoidal linear accelerative stimuli was an order of magnitude higher than that of normals Indeed, the relative importance of vestibular sensation in determination of response to accelerations has frequently been emphasized by the impairment of such response in L-D subjects (Miller & Graybiel, 1971, Graybiel & Fregley, 1966, Nashner, 1970) One might therefore surmise that the vestibular signal would predominate, especially in marginal conditions of uncertainty, which in turn points to the vestibular system itself as a likely source of the specific difficulty which appears to be associated with sensing vertical motion

Following this lead, Fig 8 shows the approximate orientations of the gravity (linear acceleration) receptor organs in man, derived from Quix (1923), Miller (1962) and Jongkees (1967) It can be seen that each organ is curved in such a way that its surface has components in all three spatial planes This feature can be better visualized in Fig 9, which is a photograph of a plastic model demonstrating the relative spatial dispositions of the planes of the gravity receptor organs The large arrow points in the forward direction and lies in the horizontal plane defined by the axes in Fig 8

It has been shown that the receptors are stimulated when the relatively dense otoliths glide over the sensory epithelia which occupy the planes seen in Figs 8 and 9 (Trincker, 1962) Each receptor cell has a direction of maximum excitability this being the direction of shear of the otolith which causes the greatest relative increase in the action potential frequency of the subsequent neuron (Lowenstein & Wersall, 1959, Flock & Wersall, 1962, Spoendlin 1966) The small arrows in Fig 9 indicate these directions of presumed maximum excitability of the receptor cells based on histological data from the guinea pig reported by Spoendlin in 1966, although some uncertainty exists as to the precise orientation of each organ relative to stereotaxic coordinates of the skull (Spoendlin, Personal Communication)

From Fig 9, it is apparent that, providing the above interpretation is correct, then vertical acceleration will generate little response from the utricular organs since these lie close to a horizontal plane and hence the small arrows have only a minimal projection in a vertical direction Rather surprisingly the model in Fig 9 indicates that even in the saccular organ there is only a relatively small projection of small arrows in a vertical direction Thus, it seems quite possible that paucity of vertical representation in the end-organ could be largely responsible for the particular difficulty attached to sensing body motion in a vertical direction, although of course central processing could also be at play

From an applied view point, the present results have an obvious bearing on the control of helicopters and VSTOL aircraft Thus from the helicopter experiments it appears that an unaided individual (i e no access to instrumentation) exposed to vertical motion without prior knowledge of its nature and direction, has no better than a 50% chance of "guessing" the direction of motion correctly and a high probability of being wildly out of phase in his assessment Furthermore from the trial using clear outside vision (but still no access to aircraft instrumentation) it seems that above some 1 500 ft, normal visual cues about vertical motion are too weak to improve the situation

Presumably in normal man-controlled flight the difficulties must be much reduced by the fact that a pilot flying an aircraft anticipates his movement according to his control action Indeed in the HCA experiments when subjects had to be shown the precise nature of their stimuli before experimentation per

formance was better than in the helicopter experiments, when subjects were falsely informed that they would be exposed to random patterns of vertical motion However, even in the HCA experiments there was no consistently reliable pattern of response, which further highlights the above implications concerning instrumentation

## ZUSAMMENFASSUNG

Die Richtigkeit subjektiver Lageempfindungen bei vertikalen, linearen Beschleunigungen wurde an Versuchspersonen mit verbundenen Augen und in aufrechter Sitzhaltung untersucht Mit Hubschraubern und einem Vertikalbewegungssimulator wurden sinusformige Vertikalbewegungen von 2–10 Sekunden Periodendauer und mit einer Beschleunigungsamplitude von 0 2–0 4 g erzeugt Die Bewegungsempfindungen wurden als standig variable Beurteilung der Vertikallage registriert Die Versuchspersonen, die uber die Art und Richtung der Bewegungsablaufe nicht unterrichtet worden waren, bemerkten wohl die Bewegung selbst, konnten aber ihre Form nur mit einer wenig mehr als zufalligen Genauigkeit erkennen Eine leichte bis massige „Turbulenz"-Simulierung brachte keine signifikanten Änderungen dieser Sachlage mit sich Eine eingehende Betrachtung der Ausrichtung der Nervenendorgane im Schadel legt die Vermutung nahe, dass das unverlässliche Orientierungsvermogen der Versuchspersonen zumindest zum Teil durch die verhaltnismassig geringe Anzahl der durch vestibulare Afferenz ubertragenen Reizinformationen uber vertikale Bewegungen verursacht wird

## REFERENCES

Benson, A J & Bodin, M A 1966 Interactions of linear and angular acceleration on vestibular receptors in man *Aerospace Med 37*, 144

Benson A J, Guedry, F E & Melvill Jones, G 1970 Response of semicircular canal dependent units in vestibular nuclei to rotation of a linear accelerat on vector without angular acceleration *J Physiol* (Lond ) *210* 475

Correia, M J, Hixson, W C & Niven, J I 1968 On predictive equations for subjective judgements of vertical and horizon in a force field *Acta Otolaryng* (Stockh ) Suppl *230* 1

Flock, A & Wersall, J 1962 A study of the orientation of the sensory hairs of the receptor cells in the lateral line organs of fish with special reference to the function of the receptors *J Cell Biol 15*, 19

Graybiel, A 1952 The oculogravic illusion *Arch Ophthal N Y 48* 605

Graybiel A & Fregly A R A 1966 A new quantitative ataxia test battery *Acta Otolaryng* (Stockh ) *61*, 292

Guedry, F E 1965 Orientation of the rotation axis relative to gravity *Acta Otolaryng* (Stockh ) *60*, 30

Jongkees, L. B W 1967 On the otoliths their function and the way to test them *Third Symposium on the Role of the Vestibular Organs in Space Exploration* (*NASA SP-152*), 307

Jongkees, L B W & Groen, J J 1946 The nature of the vestibular stimulus *J Laryng 61*, 529

Lowenstein, O & Wersall, J 1959 A functional interpretation of the electronmicroscopic structure of the sensory hairs of the crista of the elasmobranch *Raja Clavata* in terms of directional sensitivity *Nature 184* 1807

Malcolm, R 1971 *Human response to vestibular stimulation and some implications to the flight environment* Ph D Thesis, Department of Physiology, McGill University, Montreal

Meiry, J 1965 *The vestibular system and human dynamic space orientation* Sc D Thesis, Massachusetts Institute of Technology

Miller, E. F (2nd) 1962 Counter rolling of the human eyes produced by head tilt with respect to gravity *Acta Otolaryng* (Stockh ) *54*, 479

Miller, E F & Graybiel, A 1971 The effect of gravitational force on ocular counter rolling *J Appl Physiol 31*, 697

Nashner, L M 1970 Sensory feedback in human postural control *MCT 70 30* Massachusetts Institute of Technology

Niven, J I, Hixson, W C & Correia, M J 1966 Elicitation of horizontal nystagmus by periodic linear acceleration *Acta Otolaryng* (Stockh ) *62*, 429

Quix, F H 1923 L'examen clinique de la fonction des otoliths *Ann Mal Oreil Larynx 62*, No 3

Spoendlin, H 1966 The ultrastructure of the vestibular sense organ In Wolfson, R J, *The vestibular system and its diseases* U of Penn Press, Philadelphia

Trincker, D 1962 The transformation of mechanical stimulus into nervous excitation by the labyrinthine receptors *Symp Soc Exp Biol 16* 289

Walsh, E G 1961 Role of the vestibular apparatus in the perception of motion on a parallel swing *J Physiol* (Lond ) *155*, 506

— 1962 The perception of rhythmically repeated linear motion in the horizontal plane *Brit J Psychol 53*, 439

— 1964 The perception of rhythmically repeated linear motion in the vertical plane *Quart J Exp Physiol 49*, 58

Young L R. & Meiry, J L. 1968 A revised dynamic otolith model *Aerospace Med 39*, 606

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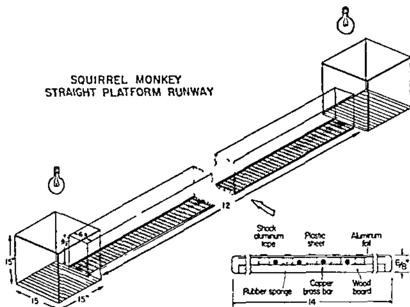



*Fig. 1.* This schematic drawing shows the structural arrangement of the present squirrel monkey platform runway (straight) device. Photocells (which are not illustrated in this figure) are installed at junctions between shuttle boxes and runway.

### *Apparatus*

The apparatus for the squirrel monkey platform runway test consists of two 15" cubical shuttle boxes connected by a 14" wide, 12' long, straight platform which is covered with a proper-sized enclosure (Fig. 1). Scrambled electric shock grids are mounted on the floor of each shuttle cage. Over each shuttle cage is a light bulb which provides a discriminating stimulus. On the floor of the platform runway, except for a 2" wide center line, 12 channels (6 channels on each side) of pressure sensor type recording system are installed. The channels are activated when a squirrel monkey steps on them. These pressure sensors are covered by a thin plastic sheet to prevent contamination, over the plastic sheet, shock aluminum tapes are installed along its longitudinal axis. These shock tapes are used during the training stage to shape the subject's performance to walk straight on the center strip. The entire device is painted black so that the subject can recognize the target (lighted) shuttle box easily. Through a small, unpainted area of the enclosure ceiling, the examiner can observe a part of the subject's walking performance.

Cumulative counters are used to record the number of channel activations in order to examine the direction and degree of dysequilibrium, an event recorder is used to print out the subject's gait performance. Crossing time for each trial run is presently measured by an electronic counter which is activated by photocell relays.

### *Subjects*

South American born squirrel monkeys (*Saimiri sciureus*) are used. Their suitability for this sort of behavioral conditioning task has been demonstrated in the squirrel monkey rail test. These subjects are one and one-half to two years of age, both males and females are used randomly. The body weights of the animals range from 550 to 750 g.

### *Procedure*

The procedure which has been developed is as follows. The subjects are first screened to determine whether they are the proper subjects for this type of psycho physical task. All animals should have an avoidance performance rate of better than 90 % for the entire trial sequence.

After such a contingency training period, the animals are subjected to 100 shaping trials

daily for five continuous training days During these 500 trials, the squirrel monkeys are trained to walk straight along the center line which connects two shuttle boxes For this purpose, squeeze-in side shocks on the platform are used

The squirrel monkeys are then processed to the preoperative baseline measurement stage Five warm-up runs are permitted on every testing day before the 10 recording trials are conducted These recordings are used to establish the preoperative baseline The tests are performed twice a week

The present criterion which determines the subject's readiness for the experimental procedure (operation, etc ) is less than five deviations (channel activations) in either direction over ten consecutive recording trials for three continuous testing days Subjects usually meet this criterion within 8 to 12 testing days

Postoperative measurements usually begin three to four days after the operation The tests are performed twice a week until the subject's bodily equilibrium performance returns to the preoperative status The manner of daily data acquisition is exactly identical to that of the preoperative stage Subsequently the animal is sacrificed by means of intracardiac perfusion and the temporal bones and related neural issues are removed and processed according to the standard preparation technique for morphological investigation

## REPRESENTATIVE DATA (LABYRINTHECTOMY)

Up to the present time seven squirrel monkeys have been assigned to the post labyrinthectomy (via retroauricular atticotomy approach) investigation After unilateral labyrinthectomy, all subjects were ataxic (in varying degrees) but their avoidance performance rate which indicates the stability of conditioning did not change More than 50 days (average 70–80 days) were required to regain the preoperative status of dynamic bodily equilibrium maintenance The data were comparable to those obtained by the squirrel monkey rail test (Igarashi et al , 1970)

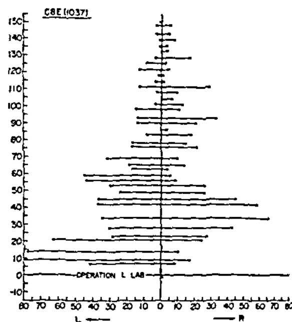



*Fig 2* This graph demonstrates the degree and direction of bodily dysequilibrium of one representative case after left labyrinthectomy The vertical axis indicates the number of days before and after the operation The abscissa demonstrates the total cumulative number of channel activations (toward subject's left or right) within ten trial runs of the day X mark on the second postoperative day indicates the extreme difficulty of performance and discontinuation of data acquisition on the day

Two cases needed more than 100 days to regain the preoperative status (Fig 2)

The direction of ataxia was toward the operated side soon after the labyrinthectomy In five cases out of seven in the present series, the direction of bodily dysequilibrium changed somewhat from one side to the other, while the number of channel activations gradually decreased from one testing day to another Therefore, in some cases, at some time after unilateral labyrinthine lesion, the direction of ataxia was toward the non-operated side The time period for one oscillation of this ataxic direction change varied, however, it was about three weeks in the present platform runway series Previously, this oscillating tendency was noticed in some of the unilaterally labyrinthectomized monkeys studied with the squirrel monkey rail test (Igarashi et al , 1970) Two other cases in the present series failed to show such a tendency

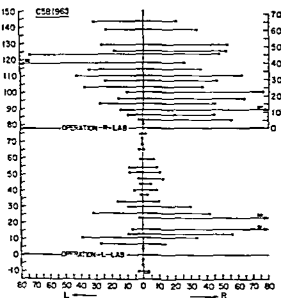



*Fig 3* This graph also exhibits the dysequilibrium status of another representative after left labyrinthectomy and subsequent right labyrinthectomy Compared to the previous case, which was demonstrated in Fig. 2, the degree of ataxia after unilateral labyrinthectomy was less and the compensation was faster in this case

Some oscillating directional change of dys equilibrium was also seen on two occasions following the second labyrinthectomy (non simultaneous bilateral labyrinthectomy) Up to the present time, however, the majority of the subjects after the second labyrinthectomy have exhibited relatively dominant directionality toward the side of the second operation It was found that recovery from dysequilibrium generally required a longer time after the second operation than after the first operation

## DISCUSSION

Compared to the squirrel monkey rail test which measures physically advanced maximum ability of dynamic equilibrium function, the present squirrel monkey platform runway test measures the degree and direction of dysequilibrium in an ordinary walk without being affected by physical training In addition, an event recorder prints out the pattern of each trial run directly on a chart thus permitting subsequent analysis of directional compensation, etc All available parameters are collected for this subsequent analysis

Platform crossing time is another important parameter which is being studied in order to analyze the equilibrium maintenance ability Reduction of ataxic degree immediately after the operation (which can be seen in the present case demonstration) is due to the fact that the squirrel monkey usually slows down to traverse the runway Thus, the crossing time should be included in the analysis of the ataxic degree

Whereas the present behavioral physical task is not a difficult one, more squirrel monkey subjects can be accepted for the experimentation as compared to the squirrel monkey rail test. Also, by utilizing the squirrel monkey rail test, the quantitation of the low end of postoperative dysequilibrium was considerably poor The severity of post-labyrinthectomy ataxia and post-utricular nerve section ataxia was "0 (maximum low score) for both categories when measured by the squirrel monkey rail test By using the squirrel monkey platform runway test, the ataxic severity will be more properly compared among different experimental categories

The results obtained from the present squirrel monkey platform runway test can be more reasonably projected toward the clinical application since the task procedure resembles the clinical balance test situation Inasmuch as the total number of labyrinthectomy cases is still limited, no statistical results are presented in this communication Representative cases only exhibit the general tendency of the post labyrinthectomy status

After unilateral labyrinthectomy, the oscillating directional change of dysequilibrium was observed in some cases with the present squirrel monkey platform runway test This tendency was previously noticed in some post labyrinthectomy cases in the squirrel monkey rail test (Igarashi et al, 1970) Similar to the bilaterally labyrinthectomized condition which was measured by the squirrel monkey rail test, equilibrium compensation after bilateral labyrinthectomy was found to be slow when measured by the squirrel monkey platform runway

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The present criterion which determines the subject's readiness for the experimental procedure (operation, etc ) is less than five deviations (channel activations) in either direction over ten consecutive recording trials for three continuous testing days Subjects usually meet this criterion within 8 to 12 testing days

Postoperative measurements usually begin three to four days after the operation The tests are performed twice a week until the subject's bodily equilibrium performance returns to the preoperative status The manner of daily data acquisition is exactly identical to that of the preoperative stage Subsequently the animal is sacrificed by means of intracardiac perfusion and the temporal bones and related neural tissues are removed and processed according to the standard preparation technique for morphological investigation

## REPRESENTATIVE DATA (LABYRINTHECTOMY)

Up to the present time seven squirrel monkeys have been assigned to the post labyrinthectomy (via retroauricular atticotomy approach) investigation After unilateral labyrinthectomy, all subjects were ataxic (in varying degrees) but their avoidance performance rate which indicates the stability of conditioning did not change More than 50 days (average 70–80 days) were required to regain the preoperative status of dynamic bodily equilibrium maintenance The data were comparable to those obtained by the squirrel monkey rail test (Igarashi et al , 1970)

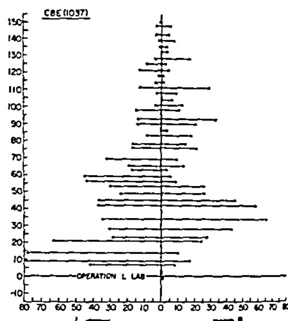



*Fig 2* This graph demonstrates the degree and direction of bodily dysequilibrium of one representative case after left labyrinthectomy The vertical axis indicates the number of days before and after the operation The abscissa demonstrates the total cumulative number of channel activations (toward subject s left or right) within ten trial runs of the day X mark on the second postoperative day indicates the extreme difficulty of performance and discontinuation of data acquisition on the day

Two cases needed more than 100 days to regain the preoperative status (Fig 2)

The direction of ataxia was toward the operated side soon after the labyrinthectomy In five cases out of seven in the present series, the direction of bodily dysequilibrium changed somewhat from one side to the other, while the number of channel activations gradually decreased from one testing day to another Therefore, in some cases, at some time after unilateral labyrinthine lesion, the direction of ataxia was toward the non operated side The time period for one oscillation of this ataxic direction change varied, however, it was about three weeks in the present platform runway series Previously, this oscillating tendency was noticed in some of the unilaterally labyrinthectomized monkeys studied with the squirrel monkey rail test (Igarashi et al , 1970) Two other cases in the present series failed to show such a tendency

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# MICROSPECTROPHOTOMETRIC DNA ANALYSIS OF MALIGNANT SALIVARY GLAND TUMOURS

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(Received 20 August, 1973)

*Abstract* Microspectrophotometric determinations of nuclear DNA quantity of Feulgen stained cells were made in six selected malignant salivary gland tumours and compared to normal salivary gland tissue and human lymphocytes from peripheral blood. Two questions of methodical importance were investigated and it was found that no errors in the quantitative DNA determination were introduced as a result of the imprint technique and that the optimal duration of acid hydrolysis in the Feulgen staining procedure was 60 min. Microspectrophotometric DNA analysis of the six malignant salivary gland tumours showed nuclear DNA quantities which exceeded that of the normal control cells irrespective of the type of tumor and degree of differentiation Furthermore, a much higher degree of intercellular variability with respect to the nuclear DNA content was found in the malignant tumour cell populations The fraction of cell nuclei with DNA values exceeding that of normal cells varied according to the type of tumour, and the incidence was small in biological low grade malignant tumours and high in high grade malignant tumours Thus the nuclear DNA content seems to be an objective parameter in grading the malignancy of salivary gland tumours

Abnormal chromosome number has been observed in malignant tumours both in experimental animals and in man (Koller, 1972), and the chromosomal abnormalities are often sufficiently pronounced to manifest themselves as an abnormal quantity of the nuclear DNA as determined by means of quantitative microspectrophotometry of Feulgen-stained cell nuclei (Leuchtenberger et al, 1954, Atkin et al, 1959, Atkin, 1964, Caspersson, 1964, Sandritter & Ritter, 1966, Böhm et al, 1971)

The salivary glands are the site of a number of different types of tumour of varying degree of malignancy Although salivary gland tumours have become more distinctly classified in recent years due to a clear histological definition, there may still be uncertainty concerning malignancy and prognosis and therefore also concerning the treatment of these tumours

In order to estimate the malignancy of salivary gland tumours in a more objective manner than by an evaluation based solely on the morphological appearance, a comparative analysis has been made between tumours with different morphological features and their cytochemical properties The aim of the present study was to investigate whether the morphologically heterogeneous group of malignant salivary gland tumours could be distinguished from normal salivary gland cells on an objective cytochemical basis and whether the degree of malignancy of the different types of tumour could be determined in the first place by using nuclear DNA quantity as cytochemical descriptive

## MATERIAL AND METHODS

Six cases of malignant salivary gland tumours were selected for analysis on the basis of the morphological features of aspirate from fine needle biopsy For the histological examination of tumours, routine histopathological techniques were employed

Immediately after surgical removal of the

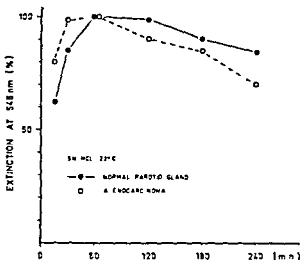



*Fig 1* Total nuclear extinction at 546 nm (amount of Feulgen positive material) plotted against time (min) of acid hydrolysis in 5 N HCl at 22 C Filled symbols and solid lines represent cell nuclei from normal parotid gland tissue and the open circles and broken lines represent cell nuclei from a poorly differentiated adenocarcinoma (case 1, Fig. 2)

tumours, imprint preparations were made from the fresh cut surface through the tumour tissue Haemacytometer glass slides were used The imprint preparations were immediately fixed in a freshly prepared mixture of ethanol and acetone (1 1) for 30 min at room temperature and thereafter stored in a refrigerator (+4°C) until the staining was performed

The DNA content of individual cell nuclei was determined after Feulgen staining by absorption measurements in a rapid scanning microspectrophotometer at 546 nm (Lomakka, 1965, Caspersson & Lomakka 1970) The acid hydrolysis in the Feulgen staining procedure was performed in 5 N HCl at 22 C The optimal duration of the acid hydrolysis was found to be 60 min (cf Results)

Freshly prepared human lymphocytes from peripheral blood were used as control cells of the staining procedure on each occasion All measured values were expressed in relation to each staining control which was given the value 2 0 C (cf Results)

Stromal cells with elongated nuclei lymphocytes and granulocytes could relatively easily be recognized in Feulgen stained preparations and were not included in the measurements Thus, only nuclei from tumour cells were randomly selected and measured

## RESULTS

Since the microspectrophotometric analysis of nuclear DNA quantity in the present study was based on the Feulgen staining reaction it was considered to be of extreme importance to test the optimal staining conditions for the different types of cell to be analysed For it has been shown that, in particular, the acid hydrolysis of DNA in the fixed cell nucleus is greatly affected by the state of the nuclear chromatin (Richards & Atkin, 1960, Sandritter et al, 1965 Kernell et al, 1971) The optimal hydrolysis conditions were therefore tested Cells from normal parotid gland tissue were compared with cells from a low-differentiated adenocarcinoma Fig 1 shows the amount of stain bound to DNA (extinction at 546 nm) as a function of hydrolysis duration The hydrolysis was performed in 5 N HCl at room temperature (22°C) The optimal duration of hydrolysis for the normal cells was between 60 and 120 min while for the tumour cells it was between 30 and 60 min 60 min was therefore considered the optimal duration of hydrolysis for all types of cell studied

Another factor of great methodological importance, which had to be considered was whether the imprint technique introduced any artefacts for the DNA determinations Since imprint preparations sometimes contain a relatively high frequency of "isolated" nuclei which might be more susceptible to the acid hydrolysis than were intact cells, imprint preparations made from mouse kidneys were compared with corresponding tissue culture preparations (primary mouse kidney epithelial cells) from parallel animals The average DNA content of the imprint preparations was 10 8 ± 1 5 (mean ± standard deviation) while for the tissue culture preparations it was 11 1 ± 1 8 (mean ± S D) Thus no significant error with respect to the Feulgen DNA determination was introduced










Fig 2 (*a*) Adenocarcinoma (*b*) Adenoid cystic carcinoma (*c*) Mucoepidermoid carcinoma Morphological appearance of imprint preparations Photomicrograph 1 400

by the imprint technique as performed in the present investigation The morphological appearance of the imprint preparations is shown in Fig 2 one poorly differentiated adenocarcinoma (Fig 2*a*) one adenoid cystic carcinoma (Fig 2*b*) and one mucoepidermoid carcinoma (Fig 2*c*)

The DNA content of the individual cell nuclei from the normal parotid gland tissue and from the six malignant salivary gland tumours is illustrated in the histograms in Figs 3 and 4 All histograms are based on randomly selected cell nuclei At each staining human lymphocytes from peripheral blood were used as control cells and the mean DNA content of these cells was given the relative value of 2 0 C which denotes the normal diploid nuclear DNA content All other DNA values are expressed in such relative lymphocyte units In this way preparations stained and measured on different occasions could be directly compared on a quantitative basis It should be pointed out however that the measured differences between lymphocyte preparations stained on different occasions were quite small under the conditions used with mean

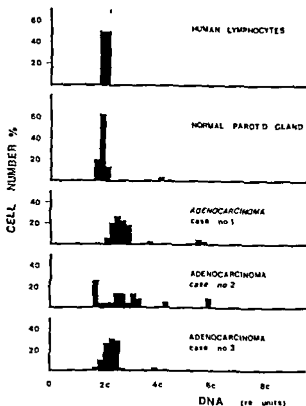



*Fig 3* Histograms of nuclear DNA content (Feulgen positive material) of individually analysed cell nuclei in the rapid scanning microspectrophotometer The cell number in each histogram class is expressed in per cent of the total number of cells analysed Except for the lymphocyte preparations in which only 30 cells were analysed between 50–100 cells were analysed in each preparation Adenocarcinomas 1 and 2 were poorly differentiated while adenocarcinoma 3 was moderately differentiated The broken vertical line represents the *upper limit of the normal diploid value*

values of 12 7, 12 8 13 1 13 1, 13 2 and 13 4 relative units of DNA on six occasions As will be seen from Figs 3 and 4 cell nuclei from the normal parotid gland tissue contain the same amount of DNA as the lymphocyte nuclei DNA values around 2 0 C i e the normal diploid amount

In the six analysed cell populations from malignant salivary gland tumours (Figs 3 and 4) from 48 % to 94 % of the cell nuclei contained more DNA than the cell nuclei from the normal parotid gland the upper limit of the normal DNA value (2 2 C) is indicated by the broken lines *in* the two figures Some of the nuclei in the tumour cell populations contained as much as 3 times the normal DNA amount As is furthermore evident from the histograms in Figs 3 and 4, the overall intercellular variability *in nuclear* DNA *amount* is considerably larger in the tumour cell populations than in the normal cell populations Despite this large DNA variability each tumour cell population was characterized by a modal DNA value in the region 2 C to 3 C, exhibited by a relatively large fraction of nuclei In cases 1, 2, 3 and 4 (three adenocarcinomas and one adenoid cystic carcinoma) this modal DNA value was found in the hyperdiploid region, significantly larger than the upper limit of the normal diploid value (2 2 C) In cases 5 and 6 (two mucoepidermoid carcinomas) the

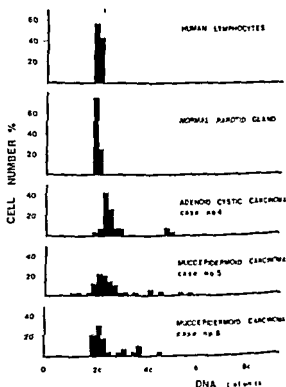



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lal DNA value was not found to be signi-ntly larger than 2 2 C, although about half the nuclei in these two cell populations tained more DNA than that value The tumour cell populations were furthermore racterized by calculating the fraction of cell lei with "normal' DNA values, defined as ies less than 2 2 C It was interesting to note : this fraction was very small in the poorly erentiated adenocarcinoma (case 1) and in poorly differentiated adenoid cystic car-oma (case 4), 6 % and 9 % of the cells respec-ly For the well differentiated mucoepider-d carcinoma (cases 5 and 6) the fraction was siderably larger, 40 % and 52 % respectively : moderately differentiated adenocarcinoma ies 2 and 3) had values in between, 33 % and % respectively An inverse relationship was s found between degree of malignancy, as ged from the morphology, and the frequency cells with "normal' DNA values

DISCUSSION

o questions of great methodological import-e were investigated in the present study stly, the optimal time of the acid hydrolysis the Feulgen staining procedure was tested l, secondly, the effect of the imprint technique quantitative DNA determinations It was nd that the hydrolysis curve of the chromatin he tumour cells differed somewhat from that the normal parotid gland cells DNA was re rapidly hydrolysed in the tumour cell clei but was also more easily lost as the lrolysis time was prolonged Since the two rolysis curves overlapped an optimal dura i of hydrolysis of 60 min could be used both normal and tumour cells It was furthermore nd that imprint preparations made from use kidney showed the same nuclear DNA tent as tissue-cultured mouse kidney cells n parallel animals This indicates that the rint technique as used in the present study, not introduce any significant errors in the ntitative determination of nuclear DNA

he microspectrophotometric DNA analysis formed in the present study showed that the cell populations from six malignant salivary gland tumours could be clearly distinguished from cell populations of normal salivary gland tissue

The six malignant salivary gland tumours were selected so as to represent different degrees of biological malignancy

The morphological features of poorly differentiated adenocarcinoma (case 1) and poorly differentiated adenoid cystic carcinoma (case 4) are connected with a high grade of biological malignancy (Eneroth, 1973, Eneroth et al, 1968), whereas the highly differentiated mucoepider moid carcinoma (cases 5 and 6) are biologically low grade malignant tumours (Eneroth et al, 1972) Cases 2 and 3 represent adenocarcinomas not so poorly differentiated as case 1

In all of the analysed malignant tumour cell populations a large fraction of cells, varying from 48 to 94 %, clearly exceeded the upper limit of the normal diploid DNA value Judging from the mitotic index, which for all tumour cell populations analysed was small (less than 0 1 %), the overall growth rates of these tumour cell populations were low Thus only a small fraction of the cells with increased nuclear DNA quantity could represent cells synthesizing DNA (S-cells) or having synthesized DNA (G2-cells) in the preparations for mitosis Therefore the large majority of the cells with increased nuclear DNA quantity represent cells in the resting phase (G0) or pre DNA synthetic phase (G1) of the cell cycle The nuclear DNA determinations thus indicate the existence of aneuploid cells with raised chromosome number since a general correlation has been found between DNA content of tumour cells and their chromosome number (Richards & Atkin, 1960) Although some tumour cell nuclei contained as much as three times the normal diploid DNA amount, the majority of them only showed moderately increased DNA quantities For the three adeno-carcinomas and the adenoid cystic carcinoma, modal DNA values in the hyperdiploid region were found suggesting the existence of aneuploid stem lines with hyperdiploid chromosome num bers For the two mucoepidermoid carcinomas, however, the observed modal DNA values were

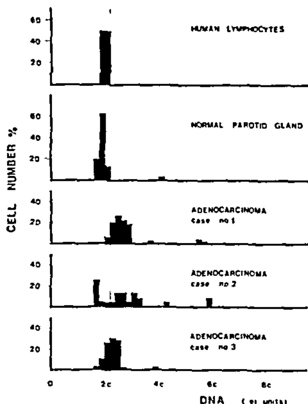



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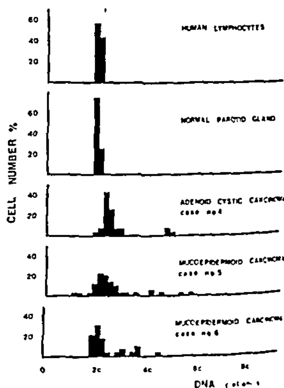



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# SPRING MECHANISMS IN THE HUMAN LARYNX

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(Received July 3, 1973)

*Abstract* Laryngeal movements are usually considered to be active in both phases of behavior cycles such as phonation, straining effort and swallowing X ray, electromyographic and cinematographic evidence is presented suggesting that these cycles begin with an active phase of muscular contraction and end with a partly passive phase of elastic restitution Elastic restitution may be a significant factor in the organization of laryngeal actions in general

The changes of the laryngeal passage in phonation, straining effort closure, and swallowing closure are organized in cycles characterized by a phase of narrowing followed by a phase of widening The succession of the phases is generally attributed to patterned activity of opposing voluntary muscles, little attention being paid to the role of adjoining elastic structures For example, in a phonatory cycle, both adduction and abduction of the vocal folds are counted as active phases and passive contributions are disregarded (Stroud & Zwiefach, 1956, Greene, 1972) One may contrast this mode of succession with the present understanding of eupneic respiration, where a cycle is seen as consisting of an active phase, the inspiratory phase, followed by a largely passive phase, the expiratory phase of elastic recoil

I submit evidence that it is the latter type of cycle—active displacement followed by largely passive restitution—that also underlies major behaviors of the larynx

Supported by USPHS Research Center Grant GM 15991

## METHODS

The evidence was obtained by means of X ray still and motion pictures, in three men and one woman, informed, consenting, and aged 30–42. Seven additional subjects were studied by cinefluorography only Electromyography was performed in two of the subjects, using a Grass electroencephalograph Concentric needle electrodes were inserted through skin wheals of 1% procaine hydrochloride as follows sternothyroid and thyrohyoid muscles, electrodes advanced down to the thyroid cartilage respectively posterior and anterior to the tubercle at the caudal end of the linea obliqua, vocalis muscle, electrode inserted through the cricothyroid ligament, cricothyroid muscle, electrode inserted at the junction of the inferior thyroid cornu *with the thyroid lamina and advanced down* to the thyroid cartilage, rectus abdominis, electrode in the craniad third of the muscle deep to the aponeurosis, external oblique muscle, entered 2 cm above and medial to the anterior superior spine of the ilium In these subjects the vertical movements of the larynx, observed externally, were recorded on 16 mm cinematographic film at 64 frames per sec The onset of a laryngeal behavior was signaled by the subject by pressing an electrical contact which actuated one of the polygraph traces and also lit a synchronizing light in the field of view of the motion picture camera

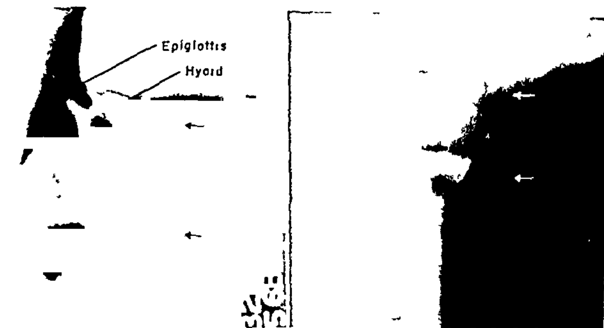



*Fig 1* Lateral radiographs of neck left, end-expiration, right, vocalizing "ah" at 256 Hz. The upper arrow points to the hyoid bone the lower arrow to the lower border of thyroid cartilage Note ascent of larynx toward hyoid bone with this vocalization

## RESULTS

I *Phonatory cycle*

(*a*) *Pre epiglottic spring* Laryngeal changes effected by phonating "ah" at 256 Hz are illustrated by Fig 1, which presents lateral radiographs of the larynx in end-expiration (left) and during the phonation (right) With phonation "ah" at 256 Hz the larynx became displaced toward the hyoid bone The pre-epiglottic body was distorted and the hyo-epiglottic ligament became elongated, as shown by the increased distance between the anterior border of the hyoid bone and the epiglottis The increase in the anteroposterior dimensions of the pre-epiglott

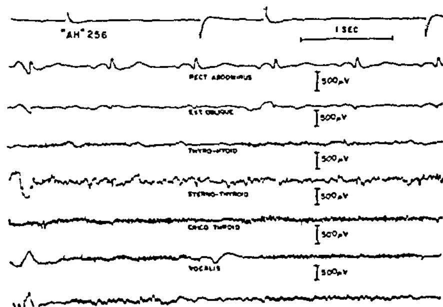



*Fig 2* Electromyogram during two successive vocalizations of "ah" at 256 Hz. The periods of phonation are indicated by the increased pen excursions in the microphone recording (lowermost trace)

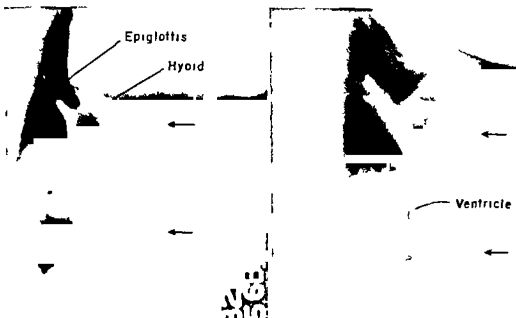



*Fig 3* Lateral radiographs of neck left end-expiration right, vocalizing ooh (u) at 256 Hz. Note descent of larynx away from hyoid bone with this vocalization

body probably also stressed the thyro-epiglottic ligament Electromyograms from the same subject during successive ah phonations are presented in Fig 2 The periods of phonation are timed by the microphone (bottom trace) Increased activity in the thyrohyoid muscle accompanied phonation but ceased with the end of phonation Activity in the sternothyroid did not increase at the end of phonation although cinematography of the neck showed that the larynx descended to its prephonatory station at this time The stretched hyo-epiglottic ligament

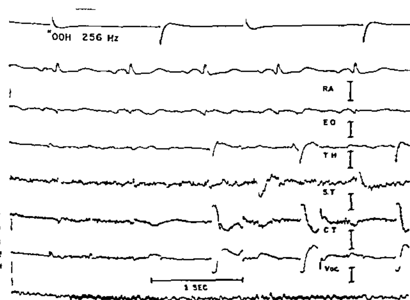



*Fig 4* Electromyograms during two successive vocalizations of "ooh" at 256 Hz.

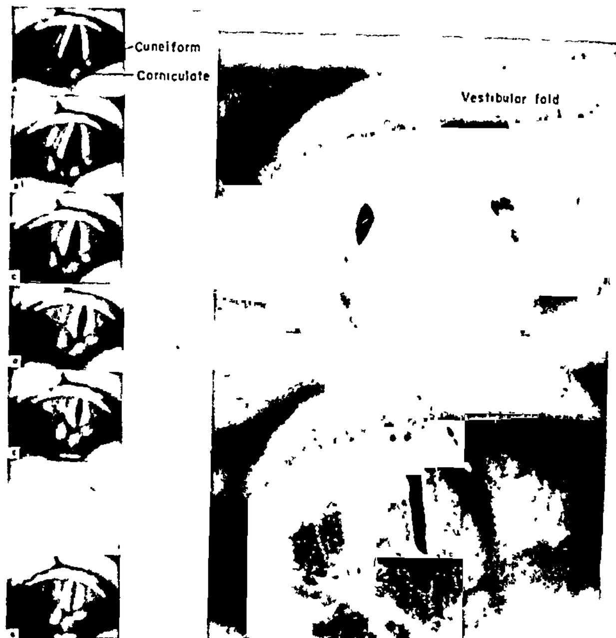



*Fig 5* Left successive frames from motion picture filmed at 16 f p s, showing adduction of vocal folds at onset of low pitch phonation (Bell Telephone) Vibration of the folds, marked by blurring is present in the last frame Compression of the corniculates against each other occurs in the course of adduction Also note concave margin of the vocal folds developed during the adduction Right two frames from a motion picture of phonatory adduction, 11/16 second apart (von Leden & Moore, 1957) Note the concavity developed at the ventricular margin of the vestibular folds

suggested by Fig 1 and the absence of increased sternothyroid activity during the phase of restitution in Fig 2 indicate that a strong element of elastic recoil contributed to the descent, including recoil of the hyo-epiglottic and thyro-epiglottic ligaments

Figs 3 and 4 show the corresponding events during phonation "ooh" (u) at 256 Hz Relative to end-expiration the phonating larynx (Fig 3 right) was displaced downward, the pre-epiglottic body became elongated and, by inference, two of its limiting ligaments, the hyo-epiglottic

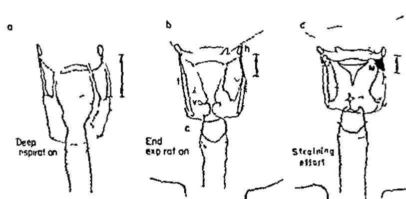



*Fig 6* Tracings of anteroposterior tomograms of neck left, deep inspiration middle end-expiration right, straining effort The pictures were al gned at the level of the hyoid bone (*h*) The outline of the thyroid cartilage lamina (*t*) is seen lateral to the vocal and vestibular folds The divergence upward of the laminae is most marked in deep inspiration and least marked during effort closure The tracings were made by a medical artist instructed by an observer both of whom were naive with respect to the questions at issue

ligament and the thyrohyoid membrane, became stretched Electromyography (Fig 4) recorded little or no increase in the thyrohyoid and sternothyroid activity during the period of vocalization and no increase in activity immediately following when the descended larynx was seen to return upward The presence of elongated ligaments during the period of sound production and the absence of electrical activity in the extrinsic muscles during the period of restitution point to participation of passive elastic recoil in returning the larynx to its prephonatory station

(*b*) *Corniculate spring* Successive frames from the Bell Telephone motion picture (16 frames per sec Fig 5, left, from above down) depict phonatory closure of the glottis As adduction of the vocal folds proceeds contact between the corniculate tubercles is observed to precede contact between the vocal processes By the time the vocal processes meet the elastic corniculate cartilages appear to overlap or are compressed against each other and form a single eminence On cinematographic projection compression was *clearly evident* When the adductor action ceased, recoil of the stressed corniculates would automatically initiate restoration of the respiratory passage at the glottis

(*c*) *Conus elasticus spring* The concavity sometimes developed by the free edge of the vocal folds during adduction (Fig 5 left) is a sign of tension tending to pull the vocal folds laterally Tension in the subjacent conus elasticus presumably contributes to this phenomenon Such tension may enter into play as an abductor force when the adductor contraction ceases The concavity sometimes seen in the ventricular margin of the vestibular fold under similar conditions (Fig 5 right) also bespeaks lateral

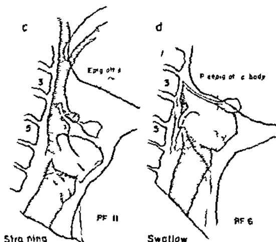



*Fig 7* Tracings from lateral radiographs of neck during straining effort closure of larynx (left) and swallowing closure (right)

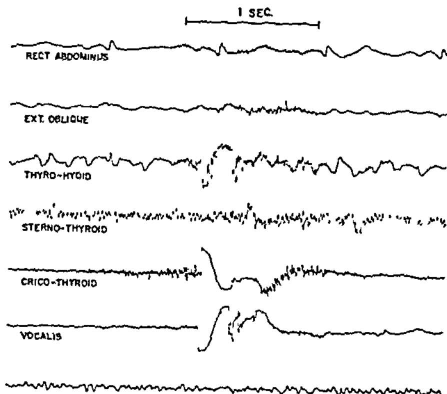



*Fig 8* Electromyograms of straining effort closure of larynx Onset of the effort is signaled by activity in the abdominal muscles (upper 2 traces) The large displacements in the 3rd 5th and 6 traces are artefacts due movement of the need caused by ascent of the larynx

tension Such tension will abduct the vestibular folds when arytenoid adduction relaxes

(*d*) *Membrana quadrangularis spring* Vocalization-associated retraction of the vestibular fold away from the vocal folds is detectable in Fig 3, where it is manifested by the vertical widening of the ventricle The widening can be interpreted in part as preventing damping of the phonating fold, and is apparently due to tension in the membrana quadrangularis created by descent of the larynx and perhaps by contraction of the ary-epiglotticus muscle (Fink, 1962) A similar effect has been demonstrated with laryngeal descent during deep inspiration (Fink et al, 1956) In either case passive elastic recoil of the stretched membrana quadrangularis will assist restoration of the pre-existing relationship

2. *Straining effort closure cycle*

(a) *Pre-epiglottic spring* In Fig 6 tracings from anteroposterior tomograms of the larynx in inspiration, end-expiration, and effort closure are aligned at the hyoid bone The characteristic ascent of the larynx with effort closure is apparent from the position of the thyroid cartilage relative to the hyoid bone In Fig 7 (left) tracing of the lateral X-ray view of the larynx during straining is presented The increased hyoid-epiglottis distance that is associated with decreased thyroid-hyoid distance is apparent This indicates that the hyo-epiglottic ligament was stretched during laryngeal ascent, and that recoil of the ligament will assist the return descent to the open configuration of the larynx

Electromyographically (Fig 8) the period of effort and of elevation of the larynx is indicated by the presence of activity in the abdominal muscles This activity is approximately coextensive with the period of thyrohyoid activity During restitution of the larynx at the end of the abdominal effort, there was little sign of electrical activity in the strap muscles Again a mainly passive recovery phase can be inferred

The vestibular folds are known to be adducted

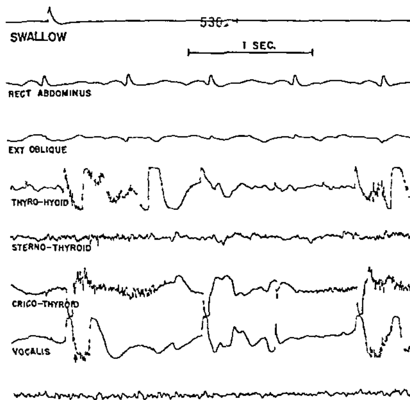



*Fig 9* Electromyograms of swallowing closure of larynx The signal in the uppermost trace was triggered by the subject s hand at the onset of swallowing The large displacements in the 4th, 6th and 7th traces are movement artefacts. The thyrohyoid trace shows electrical activation of the muscle superimposed on a movement artefact at the onset of swallowing and a movement artefact without muscle activity at the end of the swallow The beginning of a second swallowing act is seen on the right

during effort closure (Fig 6) (Lindsay, 1942), necessarily straining the elastic connective tissue external to them Restitutory retraction of the vestibular fold and cuneiform cartilage may be attributed partly to the recoil of elastic fibers in the connective tissue between the thyreo-arytenoideus and the thyroid cartilage

(*b*) *Thyroid cartilage spring* Evidence of spring action by the thyroid cartilage is detectable in Fig 6 The laminae of the cartilage are fixed below by the articulation of the inferior cornu with the cricoid cartilage but are free to bend inward and outward above The laminae diverge from below up and the angle of divergence can vary if the lamina is pliable In the subject of Fig 6 calcification of the thyroid cartilage was inconspicuous and the angle of divergence of the laminae did change with the functional state of the larynx In the tracings of Fig 6 the angle of divergence is greatest in deep inspiration (left) and smallest in the closed larynx (right)

These appearances can be explained if the sternothyroids, in pulling the laminae of the thyroid cartilage downward in deep inspiration, also tend to pull them apart, and if, during effort closure the thyrohyoids pulling the laminae upward will also tend to pull them together In either case, the strained cartilage will resume its equilibrium end-expiration configuration through its own elasticity The spring action of the thyroid cartilage has been more fully discussed elsewhere (Fink 1973)

3 *Swallowing closure cycle*

(*a*) *Pre epiglottic spring* The X-ray tracing (Fig 7, right) shows that elevation of the larynx in swallowing closure was associated with extr[illegible] deformation of the pre-epiglottic body [illegible] great elongation of the hyo-epiglottic lig[illegible] It is clear that return to the end-[illegible] configuration (Fig 1, left) will be [illegible] passive recoil of the structures st[illegible] ascent The corresponding [illegible]

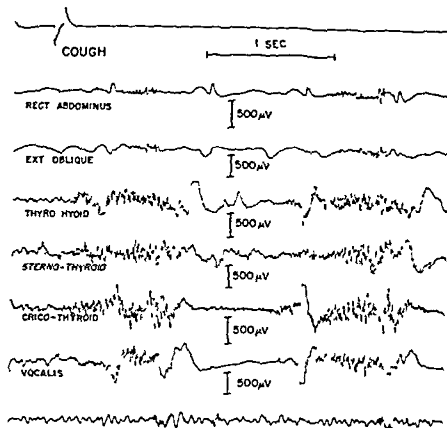



*Fig 10* Electromyograms of coughing. Note (*a*) activity in the rectus abdominus, indicating the period of effort and laryngeal ascent, (*b*) increased blackness in the microphone trace at the bottom of the figure indicating the expiratory blast of air. Cinematography showed that laryngeal descent accompanied (*b*)

(Fig 9) show increased electrical activity in the thyrohyoid and sternothyroid muscles at the start of the swallow, ending about 0 7 sec later These potentials were followed in the thyrohyoid trace by a movement artefact which cinematography identified as synchronous with the return descent phase of the laryngeal movement The decreased activity in the thyrohyoid and sternothyroid muscles at this time indicates that as regards these muscles the restitutory descent was passive

(*b*) *Epiglottic cartilage spring* Normally erect, the epiglottis usually folds over backward in the act of swallowing (Fig 7, right) apparently propelled by pressure from the tongue and pharyngeal muscles Buckling of the cartilage may be facilitated by the above noted forward pull at the line of attachment of the hyoepiglottic ligament during closure of the larynx The elasticity of the epiglottic cartilage makes it snap back passively to its equilibrium configuration at the end of the act

*4 Cough cycle*

Cinefluorographic and electromyographic (Fig 10) examination of two subjects during deliberate coughing indicated that the mechanism of laryngeal closure during a cough resembled that of effort closure However, the reopening phase, as timed by the microphone trace in Fig 10, differed inasmuch as activity in both the thyrohyoid and sternothyroid muscles was sustained at a somewhat decreased level for over 0 1 sec during this phase It appears that in cough the restitutory descent of the larynx is actively assisted

## DISCUSSION

The data permit only tentative conclusions concerning the role of passive recoil, because extrinsic muscles other than those examined could have been involved However most of these, the stylohyoid, the digastric and the mylohyoid are designated as elevators of the

larynx and are of lesser significance to the question of restitutory descent The omohyoid was the only depressor of the larynx not examined The sternohyoid was studied in one subject and its responses were similar to those of the sternothyroid

My radiographic findings corroborate those of Ardran et al (1953), and my electromyographic observations concerning the thyreohyoideus in swallowing are consistent with those of Doty & Bosma (1956), in dog, cat and monkey In these animals the thyreohyoideus activity terminated very shortly after that of the lead group of muscles of deglutition However, information expressly related to the laryngeal restitution phase on swallowing does not seem to be available (Kawasaki et al, 1964, Doty, 1968) In general, discussion of the "release" of the so-called sphincter mechanism of the larynx has been remarkably scarce (Pressman & Kelemen, 1955, Saunders, 1964 Plate IV)

The profusion of elastic tissue in and around the larynx is common knowledge (Katsumata, 1963) but its presence has not been correlated with function as clearly as has the elastic tissue in the lungs Mink (1916) many years ago ascribed the return excursion of the larynx after inspiratory descent to its elastic connections, but the role of this tissue in other laryngeal activities has received little attention Remmers & Gautier (1972) considered passive glottic reopening a possibility in feline purring

Summarizing the available evidence, one may regard the station of the laryngeal passage at end-expiration as intermediate between the wide-open, caudally displaced larynx of deep inspiration and the closed, cranially displaced larynx of effort closure and swallowing closure The intermediate partly open larynx of eupneic end-expiration represents in part an equilibrium between opposing elastic forces respectively pulling the larynx caudad and craniad There is evidence that, in addition, tonic activity of "intrinsic" laryngeal muscles operates to maintain the functional end-expiratory position of the arytenoids (Fink et al, 1956, Kotby & Haugen, 1970)

A limited analogy thus exists with the respiratory excursions of the chest, where costal and pulmonary parenchymal "springs" are in equilibrium at eupneic end-expiration (Comroe et al, 1962) Both in chest and larynx, respiratory displacements from the equilibrium are active but can be restored by passive elastic recoil In the human larynx this organization also appears to be the foundation for other behavior cycles, including phonation, effort closure, and swallowing closure As regards the intrinsic muscles, their mode of action at the glottis during phonation and respiration has long been controversial and may deserve re-examining in the light of the above postulated synergism between active and passive forces

Elastic restitution, being automatic, economizes neural energy that would otherwise be expended on timing the contraction of antagonist muscles It may possibly effect a small economy in muscular energy as well, since elastic restitution utilizes energy "stored" in structures strained during the active, agonist phase The substitution in man of a hyo-epiglottic ligament for the hyo-epiglotticus muscle of lower primates and non primates illustrates the point It is perhaps also significant that a median subhyoid air sac occurs in some lower primates in a position anatomically corresponding to that of the pre-epiglottic body of the hominoids When closed, such a sac would constitute an excellent spring

## ZUSAMMENFASSUNG

Kehlkopfbewegungen nehmen normalerweise aktiv an beiden Phasen der Funktionszyklen teil, wie z. B Phonation, Räuspern und Schlucken Aufgrund rontgenologischer, elektromyographischer und kinematographischer Untersuchungen wird angenommen, dass diese Zyklen mit einer Phase aktiver Muskelkontraktion beginnen und mit einer Phase teilweise passiver elastischer Ruckformung enden Diese elastische Rückformung ist moglicherweise ein allgemein bedeutender Faktor beim organisierten Ablauf der Kehlkopfbewegungen

## REFERENCES

Ardran, G M, Kemp, F H & Manen, L. 1953 Closure of the larynx. *Brit J Radiol 26* 497

Comroe, J H, Foster, R E II, DuBois, A B., Briscoe,

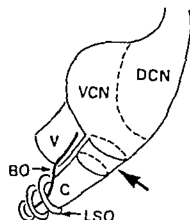



*Fig 1* The area sectioned from the cochlear nerve *VCN* = Ventral cochlear nucleus *DCN* = Dorsal cochlear nucleus *V* = Vestibular nerve *C* = Cochlear nerve *BO* = Bundle of Oorth *LSO* = Lamina spiralis ossea *Arrow points to sectioned area*

cats We were also interested in knowing whether the unimodality of the fibres described by Rasmussen & Gacek (1961) would appear in electron microscopic sections

## MATERIAL AND METHODS

*Fixation*

Three cats weighing respectively 2, 2 5 and 3 kg were anesthetized with Nembutal administered ...peritoneally, 0 5 ml per kilo bodyweight ...estruction of the left cochlea was then performed, and after 3 days they were perfused via the abdominal aorta The perfusion liquid consisted of 250 ml NaCl (pH 7 45), 500 ml paraform aldehyde 4 % in Millonig's buffer (pH 7 45) and 1 000 ml glutaraldehyde 2 5 % in Millonig's buffer (pH 7 45)

5 ml 1 % calcium chloride per litre was added to each liquid 0 5 ml Heparin was injected into the inferior caval vein before perfusion

*Preparation*

The brain and medulla were freed from the bony surroundings, and left overnight in glutaraldehyde The next day the right cochlear nerve was isolated proximal to the bundle of Oort in order to exclude the efferent fibres (Fig 1) A section of the nerve, approx ½–1 mm was cut out distal to the point where it enters the cochlear nuclei The tissue pieces were then washed in Millonig's buffer 3 × 10 min and left for 2 hours in osmium 1 % added to Millonig's buffer The blocks were then dehydrated in acetone 50 %, 70 %, 85 % and 96 %, 10 min in each liquid, and thereafter in 100 % for 25 minutes Then they were transferred to 100 % acetone and Epon aa for 15 min, and left overnight in Epon and D M P

The blocks were embedded in capsules of acetate folium and left for 12 hours in 100°C Sections 400 Å were then cut randomly on an LKB Ultrotome The sections were taken from the periphery as well as from the centre, and in different places, square to the longitudinal course of the nerve They were placed on grids and stained with lead citrate

*Photographic procedure*

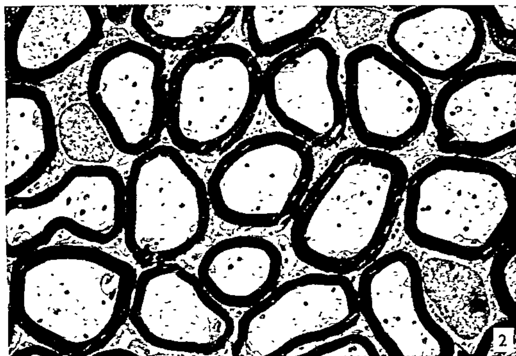
A Siemens Elmiskop was used for taking the micrographs Primary magnification was 3 000 ×, and later the photographs were enlarged × 3 Since the fibres, with exception of the outer ones, are spirally arranged through the nerve, it is impossible to present only square-cut fibres in a micrograph This difficulty was partly overcome by taking a sufficient number of pictures in different planes and by measuring a multitude of fibres An Aristo planimeter (model 1130) was employed to measure the square areas of (*a*) the axon,[1] (*b*) the axon + the myelin sheath (Figs 2 and 3)

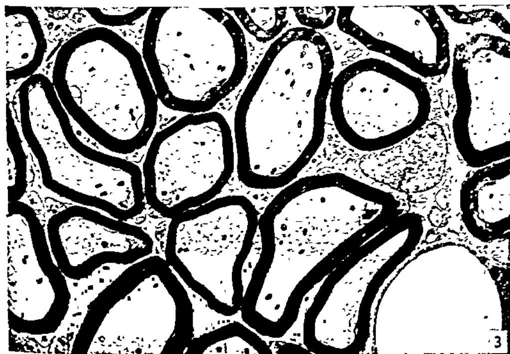
Planimetry was performed in 1 266 different areas, and by subtraction the area of the myelin sheath could also be calculated

## RESULTS

The results are shown in Figs 4–6 The figures show the areas measured in cm² of axon, axon + the myelin sheath, and myelin sheath alone, in the cochlear nerves of the 3 cats Some difference is observed between the three cases, but

[1] The expression axon is in this communication used for the area inside the myelin sheath i e the area limited by the axolemma




*Figs 2-3.* The axons surrounded by their myelin sheaths

No
Axon area
Axon area+myelin sheath
Myelin sheath

*Fig 4* Cat 1 Each column represents 222 planimetric measurements (in all, 666)

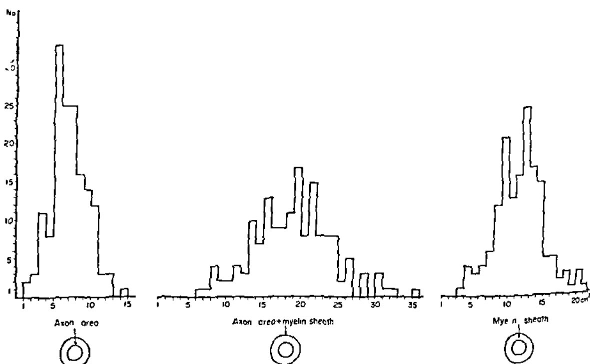



*Fig 5* Cat 2. Each column represents 156 planimetric measurements (in all, 468)

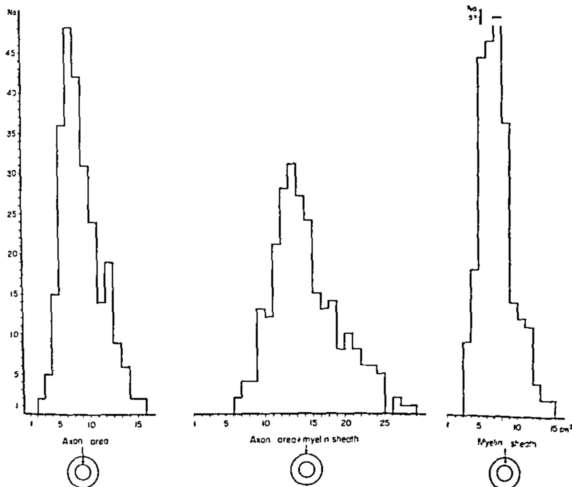



*Fig 6* Cat 3 Each column represents 255 planimetric measurements (in all, 765)

none greater than is explicable by individual variations

Thereafter, the true diameters were calculated (by computing the area with $\pi r^2$) and likewise the areas, taking into account the enlargement The two diameters and areas (maximum and minimum) are shown in Table I In this table, however, the extreme values are omitted The explanation for this procedure is that some of the pictures will show fibres which are obliquely cut, due to their spiral course in the nerve These, which are in the majority, will give an area greater than the average Other fibres will have shrunk during preparation, and their circumference will be curved inwards, thus giving a smaller area

Table II shows that if 23 % of the greater and 9 % of the smaller areas are omitted, 68 % of the fibres (axon + myelin sheath) varies between 4 and 6 μm

Table I *Minimum and maximum diameters and areas (in μm)*

| | Axon + myelin sheath | Axon | Myelin sheath |
|---|---|---|---|
| Minimum—maximum diameter, μm | 4-6 | 2-4 | 1-2 |
| Average diameter, μm | 5 | 3 | 1 5 |
| Minimum—maximum area, μm² | 14<br>25 | 4<br>15 | 6<br>19 |
| Average area, μm² | 19 | 9 | 12 |

Table II *Distribution of fibre diameters, in percentages*

| | Axon + myelin sheath | Axon | Myelin sheath |
|---|---|---|---|
| Percent fibres within minimum and maximum calculated area | 68 % (4–6 μm) | 92 % (2–4 μm) | 65 % (1–2 μm) |
| Percent above calculation | 23 % | 6 % | 18 % |
| Percent below calculation | 9 % | 2 % | 17 % |

Of course the average figures might be given with a greater variation, but Table II clearly shows that the majority of calculations (68–92–65 %) *do not differ in diameter more than* 2 μm, whether the axon and the myelin sheath, the axon alone, or the myelin sheath alone, is taken into account

## DISCUSSION

Various, although to a large extent coinciding *dimensions, have been published concerning* the calibre of the VIII nerve fibres (Kolliker, 1852 1–2 5 μm, Kolmer, 1927 3–5 μm Engstrom & Rexed, 1940 1–9 μm, and Rasmussen, 40 3–10 μm) All the measured fibres were m man Rasmussen & Gacek (1961) found he calibre in cat to vary between 1 and 8 μm A variation between 1 and 10 μm would thus cover all the previous findings These, however, were performed with a light microscope and oil immersion lenses, whereas the present authors have applied the electron-microscope The large magnification used by us gives more accurate results and the planimeter is a very reliable instrument Its main error is only 0 9 % (Hall, 1964)

Taking into consideration also the multitude of areas measured (1 266), and the large magnification (9 000 linear), the methodological errors should be practically negligible when the numbers are reduced to the true size (in μm)

The unimodal distribution of the fibres, described by Rasmussen & Gacek (1961) was also noted in the present investigation The distribution is seen from the figures 4–6 If fibres to the inner and outer hair cells had different calibres, distinct double-humped curves would have been seen This is not the case Furthermore, the results are, when diameters are considered, in accordance with those of Engstrom & Rexed (1940) and Rasmussen (1940)

## ACKNOWLEDGMENT

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## ZUSAMMENFASSUNG

Die Verfasser haben durch Planimetrie auf elektronmikroskopischen Schnitten den Diameter der Faser des Nervus Cochlearis gemessen

## REFERENCES

*Engstrom, H & Rexed B* 1940 Über die Kaliberverhaltnisse der Nervenfasern im N stato-acusticus des Menschen *Z Mikroskopischanat Forsch 47*, 448
Hall, J G 1964 The cochlea and the cochlear nuclei in neonatal asphyxia *Acta Otolaryng* (Stockh) Suppl *194*
Hildebrand C 1971 *Ultrastructural and light microscopic studies of myelinated nerve fibres in the adult and developing feline central nervous system* 166 pp *Norstedt & Soner Stockholm*
Kolmer (cit Engstrom & Rexed) 1927 In *Handbuch der mikroskopischen Anatomie des Menschen* (ed Mollendorf) vol *3*, p I
Kolliker (cit Engstrom & Rexed) 1852 *Mikroskopische Anatomie*
Rasmussen A T 1940 Studies of the VIIIth cranial nerve of man *Laryngoscope 50* 67
Rasmussen, G L & Gacek R R 1961 Fiber analysis of the stato-acoustic nerve of guinea pig cat and monkey *Anat Rec 139* 455
Sando I 1965 The anatomic interrelationship of the cochlear nerve fibres *Acta Otolaryng* (Stockh) *59* 417

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# BEHAVIORAL AUDITORY FUNCTION AFTER TRANSECTION OF CROSSED OLIVO COCHLEAR BUNDLE IN THE CAT

## II *Conditioned Visual Performance with Intense White Noise*

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*Abstract* Feline subjects were trained to respond to a visual signal in the presence of distracting background white noise The fixed ' performance ratio ' was measured by varying the intensity of the background white noise The experimental results indicated that the elimination of the crossed olivo-cochlear bundle resulted in an increase in white noise distraction of the light signal detection task in the cat Statistical analysis confirmed a significant difference between the experimental and sham groups The electron microscopic and neurohistological investigations confirmed the disappearance of the efferent nerve endings in the cochlea and that proper midline olivo-cochlear bundle sections had been made Probably one way in which the crossed olivo-cochlear bundle operates to inhibit acoustic processing is by the activation from a sensory system of a different modality

Neurophysiological studies of the olivo-cochlear bundle (OCB) after electrical stimulation or interruption of it have been conducted by many investigators After electrical stimulation of the efferent fibers, many investigators including Galambos (1956, 1960) and Fex (1962) considered the role of this system to be inhibitory in nature, or at least in part

A study by Pfalz in 1969 on the other hand described absence of function of the crossed OCB under physiological conditions in the guinea pig In our study (Igarashi et al, 1972) after transection of crossed OCB, the pure tone threshold (250–14 000 Hz) was not altered, and neither was any change found in the perceptual signal/noise ratio at the levels of 30, 50, and 70 dB above the subject's pure tone threshold Neuro-histological investigation of the brain stem confirmed that proper surgical lesions had been made in the brain stem Also, there had been clear degeneration of the efferent nerve endings around the hair cells, especially in the basal coils (electron microscopic investigation) Irvine & Webster (1972), measuring cochlear microphonic and auditory nerve action potentials in unanesthetized cats, concluded that the OCB does not function as a peripheral gating mechanism in the auditory system

Guzman–Flores & Alcaraz's experiments (1963) showed that lesion of the OCB abolishes the attenuation of cochlear evoked potentials during attentive behavior to a visual stimulus, which suggests that the auditory and visual systems are connected by the OCB

The present study was conducted in order to examine whether the crossed OCB might be activated by a non-auditory stimulus (i e, a visual signal) during exposure to intense background white noise The hypothesis of the investigation is that the OCB plays the role of gating an irrelevant auditory signal when a more significant stimulus is presented

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*Subjects*

Six screened cats were used for the present study They were clean healthy young adult cats (with no preference as to their sex) with no otological disorders Intact tympanic membranes were revealed by the otoscopic investigation and also a clean middle ear was confirmed at the time of specimen removal for electron microscopy

*Preoperative procedure*

Subjects were screened by an avoidance conditioning method using a cat rotating cage in a sound proof chamber (IAC 1202) Subjects were required to turn the cage within 5 sec after the presentation of the flashing light signal A light box was made of plexiglass covered with black paper The size was 7 cu inch A small circular hole (1/2 in diameter) in the front was the only opening of this box not covered with black paper The box was suspended about 30 from the subject (in the cage) at his head level The speaker which delivered white noise was installed at a higher level so that the box would not interfere with the noise presentation White noise (15 dB above 0 0002 dyne/$cm^2$) was constantly used to mask a faint click produced y the light The ambient light intensity of the C testing chamber approximated the level of hting outside the chamber so that light adaptation was not a factor

The subjects were further trained to rotate the cage while the light signal was on to a 90% correct response criterion The training was continued for one week Then the intensity of the light was gradually (step wise) decreased to reach the subjects positive response thresholds (dimmed light detection threshold) This particular procedure required 2 to 3 weeks There after the light intensity was increased up to the 80% correct response level and stabilized at that level which was specific for each subject Background white noise (500–15 000 Hz) was then introduced and was gradually increased in 5 dB steps until the intensity of white noise was reached at which the subjects responded to the light at only a 50% correct performance ratio (distraction level) At least six preoperative measurements were obtained

*Surgery*

Animals were anesthetized with sodium pentobarbital (30–40 mg/kg body weight) An occipital midline incision was made to expose the posterior portions of the cerebellum and brain stem The cerebellum was gently displaced upward by a malleable metal retractor The crossed OCB was cut in the floor of the fourth ventricle with a fine pick The incision was extended about 2–3 mm both rostrally and caudally from the 11 mm point anterior to the obex Three cats were assigned to the transection of the crossed OCB while the other three were randomly used for the sham control operation exposing the region retracting the cerebellum but not sectioning the crossed OCB

*Postoperative procedure*

A two week period was allowed for each subject to recuperate from surgery Immediately after this period the dimmed flashing light detection threshold for each subject (experimental and sham) was remeasured After the animals were able to respond at a mean rate of 80% or greater for four trial days at the initial 80% correct performance rate intensity the background white noise was introduced The distraction level was measured according to a method identical to that used preoperatively

After obtaining sufficient postoperative data all subjects were sacrificed for the purpose of morphological investigation After local perfusion with 2% osmium tetroxide solution (Millonig) cochlear specimens were removed and processed according to the standard procedure Specimens were embedded in Epon and ultrathin sections were cut with a Porter Blum ultramicrotome Ultrathin sections were stained with uranyl acetate and lead hydroxide and were studied under a JEM 7 electron microscope Sections obtained from at least five different areas (from basal and middle turns) of each cochlea were studied

The surgical area of the brain stem was pre

pared separately The serial cross sections of the brain stem were studied after cresylecht violet, gallocyanine or methylene-blue staining By this procedure, the depth and extent of the surgical lesions were confirmed

## RESULTS

After the two week recuperation period, the dimmed flashing light detection threshold for each subject (both in experimental and in sham groups) was remeasured and no change in it was observed The light intensity level of postoperative 80 % correct response for each subject also remained the same The sham controls required a mean of 6 0 days to reach the criterion (4 days, 80 % correct response) while the experimentals, a mean of 9 6 days

With background white noise introduction, different results were obtained between the experimental and the sham subjects The animals which belonged to the experimental group postoperatively required less intense white noise to distract them from the 80 % correct response level when compared with the preoperative state At least 72 dB noise (re 0 0002 dyne/cm²) was required to demonstrate noticeably more distraction in experimental cats All subjects in the sham operation group required slightly higher noise intensity to distract them from the 80 % correct response level The improvement

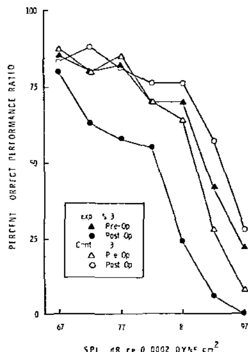



*Fig 1* This graph displays the comparison of the distracting noise level between the averages of experimentals and shams

in the performance of the sham animals is considered to be due to a training effect

Table I summarizes the pre- and postoperative results of functional testings Statistical analysis by *t* test confirmed a significant difference between the experimental and sham groups ($t$ 5 30, $p < 0\ 005$) Therefore, it is suggested from the results that the elimination of the crossed OCB resulted in an increase of white noise distraction of the dimmed flashing light signal detection task in cats Figure 1 graphically displays that less intense noise distracts visual performance in experimentals postoperatively

Electron microscopic investigations confirmed the disappearance of the efferent nerve endings around the hair cells, especially the basal coils of the experimental subjects (Fig 2) Also, the surgical lesions in the brain stems were neurohistologically confirmed to have been properly made to interrupt the crossed OCB The midline sections extended about 2 0 mm (both rostrally and caudally) beyond the edges of the facial colliculi (Fig 3)

Table I *This table demonstrates the preoperative and postoperative distracting white noise level of the visual task*

The statistically significant difference was found in the required noise level between the experimental (X) and sham (S) groups See text

| Subject | Noise level (dB re 0 000 2 dyne/cm²) | | |
|---|---|---|---|
| | Pre-Op | Post-Op | Difference |
| XA | 95 5 | 84 8 | 10 7 |
| XB | 89 0 | 84 8 | − 4 2 |
| XC | 90 0 | 78 5 | 11 5 |
| SA | 88 0 | 90 8 | 2 8 |
| SB | 92 0 | 96 5 | 4 5 |
| SC | 89 0 | 92 8 | 3 8 |




*Fig 2* The electronmicrograph demonstrates the disappearance of efferent nerve endings at the inferior portion of the outer hair cells (*arrows*) A, afferent nerve endings This specimen was obtained from the upper basal turn × 7 000

## DISCUSSION

In 1971 Borg measured the response of the acoustic middle ear reflexes in unanesthetized and unrestrained rabbits Chronic lesions were made to interrupt the crossed OCB Only inhibitory influences were found It was suggested that the inhibitory influences observed may be due mainly to tonic spontaneous activity from the efferent fibers In an experiment of the cross modality between middle ear muscles and the visual system, Simmons et al (1959) trained feline subjects to expect a loud noise when a light appeared Middle ear muscles were cut on one side Evidence of successful training was given by demonstrating a conditioned response of the middle ear muscles on the uncut side when the light alone was presented The experimental results demonstrated that middle ear muscle excitation can be altered by a visual stimulus

Inasmuch as in the present study the middle ear muscles were not cut, it is not known whether the results obtained are due to the inhibitory

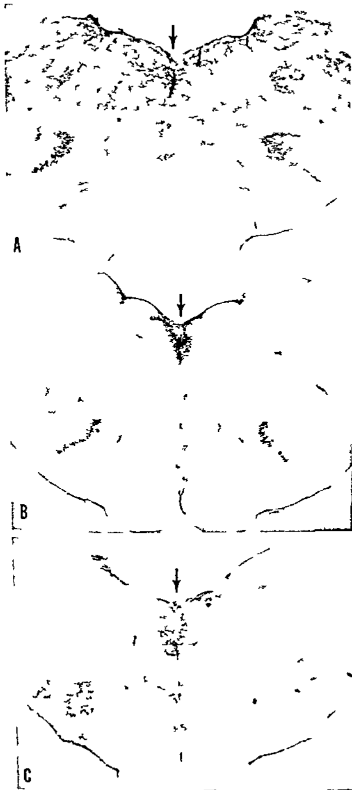



*Fig. 3* Three transverse sections of the brain stem demonstrate the rostro-caudal distribution of midline cut lesions (*arrows*) A about 2 mm rostral to the level of the facial genu B at the level of the facial genu C about 2 mm caudal to the level of the facial genu Cresylecht violet stain 10

mechanism at the peripheral end organ level, or to a functional inhibition at the level of the middle ear muscles It is noteworthy, however, that in our previous study (Igarashi et al, 1972) with white noise of comparable intensity, no effect was noted after crossed OCB transection upon increased white noise masking In addition, the existence of olivary complex–cochlear nucleus connections cannot be neglected In any event, however, it was observed that the midline lesion has some effect when a subject responds to a visual stimulus under an intense background white noise, even though the lesion may affect in an unspecific way in animals' behavioral responses

Harrison & Irving (1966), after studying the monkey, bat, dolphin, guinea pig, rat, mouse, and hedgehog, described how the medial superior olive appeared to be a part of the auditory system which was in some way related to vision The medial superior olive was well developed in all diurnal animals and in nocturnal animals with good vision such as cats, and in animals with well developed eyes The medial superior olive was probably not concerned with auditory locali zation in the psychophysical sense, but was probably concerned with the movement of the and eyes in the direction of the sound in Thus, this area may be a contact between auditory and visual systems

In addition to subcortical connections, connections may exist between visual system and the insular temporal cortex which is suspected as an auditory (integratory) cortex (Desmedt, 1960) In regard to the corticofugal connections, according to Rasmussen (1964) two cortical fiber connections are the corticogeniculate fibers and the fibers to the nuclei of the brachium and the inferior colliculus Some fibers from the nucleus of the inferior colliculus enter the superior olivary regions, however the functional significance of these fibers is not known

In 1959 Hubel and others found that while they were recording unit responses from the auditory cortex in unrestrained and unanesthe tized cats, a population of cells appeared to be sensitive only when the subject paid attention to the tone source This experimental result very clearly indicated the importance of the functional investigation in unanesthetized subjects, and also suggested that paying attention can evoke or at least assist auditory neural function within a psychob ological unit

It is known that in higher mammals (cats) the OCB has about 500 fibers which in turn supply 40 000 efferent nerve endings (Spoendlin, 1966) As such it is suggested that the OCB function, if any, is probably relatively general for the in hibition of auditory input Probably one way in which the OCB operates to inhibit acoustic processing is by the activation from a sensory system of a different modality

## ACKNOWLEDGMENT

Appreciation is expressed to Mrs M Lewis Mrs E Mar b'ey and Mrs K Cummings for their technical assistance

## ZUSAMMENFASSUNG

Versuchskatzen wurden trainiert auf sichtbare Signale zu reagieren wahrend im Hintergrund ablenkender, weisser Larm zu horen ist Das bestimmte Verhältnis ihres Verhaltens wurde durch die Veranderung der Inten sität des weissen Larms im Hintergrund gemessen Die Versuchsresultate bewiesen dass die Katze ohne das ge kreuzte OCB [Olivo Cochlea Bundel] durch den weissen Larm mehr von ihrer Aufgabe abgelenkt wird auf die Lichtsignale zu reagieren Eine statistische Analyse durch t Versuch bestatigte einen bedeutenden Unterschied zwi schen Versuchs und Scheingruppen Die elektronen-mikroskopischen und neurohistologischen Versuche be stätigen das Verschwinden der austragenden Nervenenden in der Cochlea und dass richtige, mittel lineare OCB Sektionen gemacht wurden Eine Moglichkeit in welcher das OCB so funktioniert dass es den Horprozess kon trolliert besteht wahrscheinlich in der Aktivierung des OCB durch ein Empfindungssystem mittels eines anderen Verfahrens

## REFERENCES

Borg E 1971 Efferent inhibition of afferent acoustic activity in the unanesthetized rabbit *Exp Neurol 31* 301

Desmedt J E 1960 Neurophysiological mechanisms controlling acoustic input In *Neural mechanism of the auditory and vestibular systems* (ed G L Rasmussen & W F Windle) p 152 C C Thomas Spring field Ill

Fex J 1962 *Auditory activity in centrifugal and centri* petal cochlear fibres in cat *Acta Physiol Scand* Suppl *189*

Galambos, R 1956 Suppression of auditory nerve activity stimulation of efferent fibers to cochlea *J Neurophysiol 19*, 424
— 1960 Studies of the auditory system with implanted electrodes In *Neural mechanism of the auditory and vestibular systems* (ed G L Rasmussen & W F Windle) p 137 C C Thomas, Springfield, Ill
Guzman Flores, C & Alcaraz, M 1963 Funcion de la cintilla olivo coclear en el fenomeno de distraccion a estimulos acusticos Nota preliminar *Bol Inst Estud Med Biol 21*, 87
Harrison, J M & Irving, R 1966 Visual and nonvisual auditory systems in mammals *Science 154*, 738
Hubel, D H, Henson, C O, Rupert, A & Galambos, R 1959 Attention" units in the auditory cortex *Science 129*, 1279
Igarashi M., Alford, B R, Nakai, Y & Gordon, W P 1972 Behavioral auditory function after transection of crossed olivo-cochlear bundle in the cat I Pure-tone threshold and perceptual signal to-noise ratio *Acta Otolaryng* (Stockh) *73*, 455
Irvine, D R F & Webster, W R 1972 Studies of peripheral gating in the auditory system of cats *Electroenceph Clin Neurophysiol 32*, 545
Pfalz, R K 1969 Absence of a function for the crossed olivo-cochlear bundle under physiological conditions *Arch Klin Exp Ohr Nas Kelhkopf heilk 193*, 89
Rasmussen, G L 1964 Anatomic relationships of the ascending and descending auditory systems In *Neurological aspects of auditory and vestibular disorders* (ed W F Fields & B R Alford), p 5 C C Thomas, Springfield, Ill
Simmons, F B, Galambos R & Rupert, A 1959 Conditioned response of middle ear muscles *Amer J Physiol 197*, 537
Spoendlin, H 1966 *The organization of the cochlear receptor* S Karger, Basel and New York

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# THE RELEASE OF ACETYLCHOLINE (ACH) BY THE CROSSED OLIVO COCHLEAR BUNDLE (COCB)

C H Norris and P S Guth

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(Received March 20, 1973)

*Abstract* A method has been developed to detect acetylcholine like activity in the perilymph of guinea pigs The acetylcholine like activity in the guinea pig perilymph is not significantly increased by moderate acoustical stimuli but it is significantly increased when the crossed olivo-cochlear bundle is tetanically stimulated The criteria for identifying acetylcholine as the chemical mediator at the crossed olivo-cochlear nerve/hair cell junction are now largely fulfilled

The possibility that the junctional transmission between the olivo-cochlear nerve endings and the cochlear hair cells is chemically mediated has been suggested by many authors (Gisselsson, 960, Fex 1962, Amaro et al, 1966, Katsuki, 6, Daigneault & Brown, 1966, Bobbin & nishi, 1971) Of all the neurohumors said to perate at this junction, the candidacy of acetylcholine (ACh) has received the most experimental support The strict localization of the enzyme, acetylcholinesterase (AChE), to the crossed olivo cochlear bundle (COCB) endings demonstrated by Churchill et al (1956) initiated the idea that ACh may be the transmitter at these endings Since that time many other investigators have studied the histochemical localization of AChE to the cochlea using both light microscopy and electron microscopy An excellent summary of these findings is given by Iurato et al, 1971 However acetylcholinesterase is a rather ubiquitous enzyme and its presence alone is not sufficient proof that a particular junction is cholinergic in nature

The identification of a neurohumor depends *upon the substantiation of several criteria* (Eccles, 1964) A typical list as given by both Werman (1966) and Phillis (1970) is as follows 1) *Release* During stimulation, the candidate substance should be demonstrable in the extracellular fluids collected from the region of the activated synapse 2) *Duplication* Exogenous application of the candidate substance to the post synaptic membrane should mimic the action of the synaptically released transmitter 3) *Pharmacological* Pharmacological agents which interact with the putative transmitter at other biological sites should have analogous interactions at the synapse under consideration, depending upon the properties of the receptors 4) *Termination* An inactivation system should be demonstrable 5) *Synthesis* There should be present the necessary structural and enzymatic mechanisms for the manufacture and storage of the transmitter

The criteria of duplication, termination and synthesis have been conclusively fulfilled for the acetylcholine like activity at the COCB hair cell synapse That is, Bobbin & Konishi (1971) reported that ACh mimics COCB stimulation Churchill et al (1956) demonstrated the presence of the terminating enzyme acetylcholinesterase, and Jasser & Guth (1973) demonstrated the presence of the ACh synthesizing enzyme choline

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acetyltransferase The criterion of pharmacological interactions is supported by some data and can be questioned by other data Apparently damaging to the fulfillment of the pharmacological criterion is the finding of Desmedt & Monaco (1960) that strychnine, which was not known to be an anticholinergic, blocks the effects of COCB stimulation The work of McKinstry & Koelle (1967) later showed that strychnine could inhibit the release of acetylcholine Thus the strychnine block may be supporting rather than detracting evidence Also *damaging to the pharmacological* criterion is the fact that Desmedt & Monaco (1961) were unable to block OCB effects with dihydro-B erythroidine Sohmer & Feinmesser (1963) could not measure changes in cochlear microphonics (CM) or gross eighth nerve action potentials (AP) by using atropine and physostigmine Tanaka & Katsuki (1966), using iontophoretic means, could not relate the effects of various cholinergic and anticholinergic agents to the olivo cochlear inhibition of AP caused by electrical stimulation of the COCB It is possible that route of drug administration, or technical difficulties could account for the above negative results or it is possible that the hair cells have a new or modified type of cholinergic receptor

However, the cholinergic hypothesis is gaining additional support as methods become more refined and drugs are applied directly into the cochlear fluids rather than systematically For example, Guth & Amaro (1969) were able to demonstrate a classic hemicholinium-3 effect on the activity of the COCB, Fex (1968) was able to block COCB activity with d-tubocurarine, Galley et al (1971) blocked the AP inhibition caused by COCB stimulation by applying gallamine triethiode directly to the scala tympani, and Bobbin & Konishi (personal communication) were able to block COCB activity with a fairly large series of *standard* anticholinergic drugs Finally, the criterion of release which may be considered the keystone criterion, is supported only by the preliminary discussions of Fex (1968) and Whitfield (1967) in the September, 1967 Ciba Symposium on Hearing Mechanisms in Vertebrates Both mentioned pilot experiments which appeared to be positive for ACh but to this date neither of them has published the results of his experiments Therefore, the present work was done in order to determine whether or not the criterion of release could be substantiated Of all the criteria, this seems to be the most important to test and a positive result here could provide the strongest support for the cholinergic hypothesis

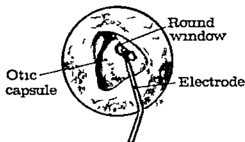



*Fig 1* Post auricular approach to the round window showing placement of the recording electrode

## METHODS

*Surgical technique*

Young guinea pigs weighing between 150 and 200 grams, of either sex, are anesthetized with pentobarbital (25–30 mg/kg i p) At the start of surgery cannulas are introduced into the trachea, carotid artery and external jugular vein for purposes of artificial respiration, blood pressure measurements and drug administration, respectively Next, the structures around the ventral portion of the bulla are cleared away and the bulla is opened, exposing the middle ear and the otic capsule At this time both the stapedius and tensor tympani muscles are separated from their respective insertions After opening the bulla the animal is turned in a specially designed head holder and a post auricular approach is made to the round window A silver/silver chloride *monitoring electrode is carefully placed on* the round window membrane and cemented in place with zinc dental cement (Fig 1) This electrode is used to measure the response of the cochlea to an audible click or to a tone burst Next, the skull of the animal is opened from

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*Fig 4* Bioassay recording 0 04 ml of perfusate collected during COCB stimulation elicits ileum contractile responses equivalent to 0 3 ng of acetylcholine Three hundredths (0 03) μl no 1 refers to perfusate samples Numbers followed by ng refer to standard doses of acetylcholine in nanograms

direction from the correct location little or no COCB effect could be obtained

After satisfactory stimulating electrode placement has been obtained, push pull glass micropipettes, mounted on a micro-positioner and filled with artificial perilymph are inserted through the holes in the otic capsule The artificial perilymph is composed of NaCl, 140 mM, KCl 5 mM, $CaCl_2$, 5 mM, eserine sulfate $10^{-5}$ M and albumin, 40 mg %. It is gassed for 10 min with a 5 % $CO_2$ 95 % $O_2$ mixture and the pH is adjusted to 7 2 with $NaHCO_3$ The pipettes are connected to a continuous perfusion withdrawal pump in such a manner that artificial perilymph is introduced into scala vestibuli and simultaneously collected from scala tympani The rate of perfusion is set at 1 97 μl per min and in typical experiments each perfusion is performed for periods of 70 to 90 min Perfusions are performed with and without stimulation of the COCB and with and without sound stimulation The collected perfusates are then bioassayed for the presence of neurohumors

*Bioassay technique*

During the assay procedure, a contractile response is periodically elicited from a piece of guinea pig ileum mounted in a small smooth muscle bath. The procedure is as follows The flow of Tyrodes through the bath is clamped a standard concentration of agonist (i e acetylcholine) is injected into the bath solution covering the ileum 10 sec later the Tyrodes flow is unclamped The procedure is repeated at one-minute intervals and during each such procedure, the ileum will undergo a contraction followed by a relaxation

Initially, an ileum will be rather insensitive requiring large concentrations of agonists (20–40 ng of acetylcholine) but as time progresses and the concentrations of agonist injected are systematically decreased, it can become very sensitive (e g responding to an injection 0 1 ng acetylcholine) In performing the actual assay, bath injections of stock artificial perilymph are made in volumes equal to those of the perfusate to be assayed This control is necessary since the ileum at times contracts in response to the stock artificial perilymph. Resting and then restimulating the ileum with standard agonists will sometimes eliminate this problem, but at other times a new piece of ileum must be installed in the bath In all cases each injection (artificial perilymph or perfusate) is spaced in between an injection of standard acetylcholine made up in artificial perilymph The known concentrations of acetylcholine in the standard injections are selected to cause ileum contractions of magnitudes approximately equal to those caused by the perfusates being assayed A standard six point bioassay with interspersed standard responses higher than equal to, and lower than the responses to an unknown was not usually possible since the volume of perfusate collected during the perfusion experiments was insufficient to make more than two or three bath injections Usually one perfusate injection was used to find the range of ileum contractile response, then a second injection was used to approximately quantify the response, and a third injection was reserved for checking with blocking agents such as acetylcholinesterase or atropine sulfate A typical bioassay recording is shown in Fig 4 in which 0 04 ml of collected perfusate caused an ileum response which is equivalent that caused by 0 3 ng of ACh. Responses of an ileum to acetylcholine can be selectively blocked by adding 40 μg/liter of atro-

*Fig 5* Bioassay recording Ileum contractile activity in 0 1 ml perfusate collected during COCB stimulation is reduced by incubation with acetyl-cholinesterase One hundredth (0 01) µl no 21 refers to perfusate samples Numbers followed by ng refer to standard doses of acetylcholine in nanograms

pine to the Tyrodes bathing solution or by adding acetylcholinesterase to the active perfusate The contractile activity of the perfusate samples should be blocked or significantly diminished after such manœuvres if that activity was originally caused by acetylcholine Fig 5 is a recording showing how an ileum contractile response elicited by collected perfusate is completely blocked the addition of acetylcholinesterase to the ple

*istology*

Upon completion of the perfusion experiments in some of the animals, the temporal bones were prepared for histological examination by the surface preparation technique of Engstrom et al (1966) After some of the other perfusion experiments, the ears were prepared by the method of Guild (1921) as modified by Schuknecht (1953) The goal of these examinations was to determine if the perfusion technique had caused any observable cochlear damage

*Preliminary experiments*

More than thirty preliminary experiments were performed During these experiments, several of the parameters in the protocol were varied in order to achieve the optimum results For example, during preliminary experiments, the rate of application of the tetanic stimulation to the COCB was varied in order to test the possibility that the nerve could be fatigued It was important to measure this possibility since the stimulation perfusions had to be carried on for more than 1 hour in order to collect enough perfusate Fatigue was measured as a loss of AP inhibition Optimal AP inhibition was usually equivalent to a 15 to 20 dB decrease in click intensity If the COCB was stimulated at a rate of two tetanic stimulations per second, fatigue (2 to 6 dB of equivalent click intensity) could be seen within the first 5 sec and the inhibitory effects of COCB stimulation were completely dissipated within 10 to 15 sec After a short period of rest (30 sec) the inhibition was restored At tetanic stimulation rates of one each 2 sec, fatigue was seen after 35 min, however, tetanic stimulations of the COCB applied once each 5 sec did not produce any measurable fatigue for 1 hour or more and this was the rate used for most of the experiments

In some of the early experiments, the calcium concentration in the artificial perilymph was 2 8 mM, but in most of the later experiments the calcium concentration was increased to 5 6 mM in an effort to obtain an increased yield of neurohumor Higher calcium concentrations, up to 10 mM, were also considered, but when the artificial perilymph containing these concentrations was tested on the smooth muscle assay preparation it seemed to cause excessive spontaneous activity which prevented that particular assay preparation from being used again

In another set of three experiments the cochlea was perfused with artificial perilymph containing known quantities of ACh Bioassay of the collected perfusate showed that better than 90% of the ACh was recovered in each case It was therefore concluded that the perfusion technique did not appear to cause any substantial losses in acetylcholine that might have been in the perilymph Attempts were also made to store recovered perfusate in the freezer Perfusate stored over-night lost up to 50% of its activity

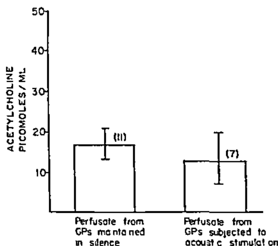



*Fig 6* Background acetylcholine-like activity released into perilymph Differences are not statistically significant No electrical stimulation of COCB was used in either case Click strength was 1 volt 80 msec pulse applied to TDH TDH 39 earphone

and perfusate stored for several days lost more than 50 % of its activity Storage in soft glassware also seemed to cause the perfusates to lose activity so that in all of the later experiments the perfusates were collected and stored only in hard glassware

After the first preliminary experiments established that the collected perfusates exhibited ileum contractile activity a protocol was initiated wherein all active perfusates were tested with either acetylcholinesterase or with atropine In all cases tested, the contractile activity was completely blocked In a few cases enough perfusate was collected so that both tests could be applied and again the ACh like contractile activity was blocked The concentration of atropine used did not block equivalent ileum contractile responses elicited by histamine bradykinin and 5 hydroxy tryptamine

## RESULTS

Data from 43 successful experiments are given in Figs 6 7 and 8 Fig 6 compares the results from seven perfusion experiments during which the animals were subjected to free field clicks from the TDH 39 earphone and eleven other perfusion experiments during which the animals were maintained in the relative silence of a sound dampened chamber In no case was the COCB stimulated The clicks were applied at the rate of two per second and were derived from the application of a 1 V, 80 msec square wave pulse to the earphone All bioassay results are reported in terms of the picomoles of standard acetylcholine per milliliter which equivalently stimulate the ileum An analysis of variance justified the use of the pooled *t* test which then demonstrated that no significant difference existed between the means of the two sets of data (perfusion during silence vs perfusion during click) It was therefore concluded that there was no significant difference between the amount of acetylcholine like activity in the perfusates collected when the animals were maintained in silence or when the animals were subjected to acoustic clicks

Fig 7 compares the results from twelve experiments in which control perfusions were

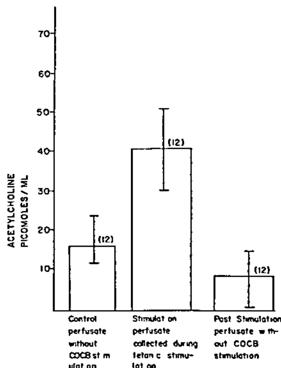



*Fig 7* Acetylcholine-like activity released into perilymph Results are significantly different $p < 0.001$ Tetanic stimulation – 40 shocks 1 msec wide and 200 $\mu$A in amplitude given at the rate of 400 shocks second

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fluid spaces of the cochlea? 2) If present, is this neurohumor acetylcholine? 3) If present, is the quantity of released neurohumor correlated with COCB stimulation? The experiments depicted in Fig 6 were carried out because Martini (1940) reported that acoustical stimulation produced ACh-like activity in perilymph collected from pigeons Our results, on the other hand, indicate that click stimulation does not cause a detectable rise in perilymph ACh-like activity This incompatibility between our results and Martini's may be based on methodological or species differences

The significant increase in the acetylcholine-like activity released into the perilymph during COCB stimulation lends strong credence to the cholinergic hypothesis but the absolute amount of neurohumor collected is somewhat disappointing Eccles (1964) gives an estimate of the acetylcholine output from a single cholinergic fiber as being between $5 \times 10^{-17}$ and $3.5 \times 10^{-16}$ grams/impulse Assuming $1 \times 10^{-16}$ grams/impulse, 40 impulses per tetanic stimulation, 40 000 fiber endings, and a 90 min perfusion period, then the expected accumulated output of ACh would be 956 pmoles of acetylcholine per perfusion Actually an average stimulation perfusion yielded only 5 pmoles of acetylcholine-like substance One of the many possible explanations for this low yield is that the neurohumor is prevented from reaching the perilymph by the basilar membrane In light microscope preparations the basilar membrane appears as an effective seal between the organ of Corti and the scala tympani and this has been used as an argument in favor of the existence of cortilymph Such a membrane barrier could easily explain why Bobbin & Konishi (1971) had to use large concentrations of drugs and why the pharmacological evidence did not begin to yield strong positive results until steady perfusion techniques had been utilized This membrane barrier could also be the cause of the high recovery seen with perfusions of known concentrations of acetylcholine The new results presented herein, plus the evidence of many of the previous pharmacological investigations wherein differing results were obtained depending upon whether or not the drugs were given systematically or intracochlearly, therefore imply the existence of blood/cochlea and hair cell/perilymph barriers These barriers must be taken into account when considering the distribution of the therapeutic and experimental drugs to the inner ear The experimental results make it most likely that some of released neurohumor does move from the COCB/hair cell junction through the cortilymph and basilar membrane into the perilymph, but some released neurohumor may also diffuse back along the nerve fibers to the habenula perforata and then into the scala tympani It is unlikely that the released neurohumor diffuses into the scala vestibuli since it must pass the reticular lamina, the tectorial membrane, the endolymph environment of the scala media, and Reissner's membrane before reaching the scala vestibuli In addition, the large concentration of acetylcholinesterase along the presynaptic nerve terminals will hydrolyse much of the released neurohumor before significant diffusion is possible The concentration of eserine which may have passed from the perfusion fluid through the basilar membrane may not have been sufficient to block this acetylcholinesterase

The identification of ACh as the COCB/hair cell transmitter has possible significance with regard to the hypothesized transmitter existing between the hair cells and the afferent nerve terminals It is unlikely that both the afferent and efferent transmitter substances are the same, since there is a distinct possibility of an interaction of the two in the cleft below the hair cells and ACh has never been shown to enhance afferent nerve activity Additionally, Jasser & Guth (1972) found an almost complete abolition of choline acetylase activity when the COCB degenerated Finally, the choline esterase activity is almost completely localized presynaptically on the COCB endings (Iurato, 1971)

In conclusion then, an ACh-like substance is present in the fluid spaces of the cochlea and its concentration is significantly increased during COCB stimulation Therefore, the criteria for identifying acetylcholine as the chemical media-

tor at the crossed olivo-cochlear nerve/hair cell junction are now largely fulfilled.

## ACKNOWLEDGMENT

The authors gratefully acknowledge the assistance of Ms Marion Stockwell who carried out most of the bioassays

## ZUSAMMENFASSUNG

Eine Methode zum Nachweis von acetylcholine-ahnlicher Aktivität in der Perilymphe von Meerschweinchen ist entwickelt worden Diese Aktivitat wird durch mässige akustische Erregung unbedeutend erhoht Sie nimmt aber durch tetanische Erregung vom gekreuzten Olivo Cochlea-Bündel bedeutend zu Die Kriterien, um Acetylcholine als den chemischen Vermittler der Verbindung der gekreuzten Olivo-Cochlea Nerven-Haarzellen Verbindung auszusehen, sind nun grossenteils gegeben

## REFERENCES

Amaro, J , Guth, P S & Wanderlinder, L 1966 Inhibition of auditory nerve action potentials by acetyl-

Churchill, J A , Schuknecht, H F & Dorand, R 1956 Acetyl-cholinesterase activity in the cochlea *Laryngoscope 66*, 1

Daigneault, E A & Brown, R D 1966 Acetylcholine suppression of the $N_1$ component of round window recorded cochlear potentials *Arch Int Pharmacodyn 162*, 20

smedt, J E 1962 Auditory-evoked potentials from cochlea to cortex as influenced by activation of the efferent olivo-cochlear bundle *J Acoust Soc Amer 34*, 1478

Desmedt, J E & Monaco, P 1960 Suppression par la strychnine de l effet inhibiteur centrifuge exerce par le faisceau olivo-cochleaire *Arch Int Pharmacodyn 129*, 244

— 1961 Mode of action of the efferent olivo-cochlear bundle on the inner ear *Nature 192*, 1263

Eccles, J C 1964 *The physiology of synapses* Academic Press, New York

Engstrom, H , Ades, H W & Andersson, A 1966 *Structural pattern of the organ of Corti* Almqvist & Wiksell, Stockholm

Fex, J 1962 Auditory activity in centrifugal and centripetal cochlear fibers in cat *Acta Physiol Scand 55*, Suppl 189

— 1964 Crossed cochlear efferents activated by sound through both ears *Acta Physiol Scand*, Suppl *213*

— 1968 Efferent inhibition in the cochlea by the crosse olivo-cochlear bundle In *Hearing mechanisms in verte brates* (ed A V S Dereuck & J Knight), p 16 Little Brown & Co , Boston

Galley, N , Klinke R Pause M
*The effect*
cochlea *Pf*

Gisselsson, L
choline injected into the endolymphatic space *Act Otolaryng* (Stockh ) *51*, 636

Guild S R 1971

Gu

8, 49

Iurato, S , Luciano, L , Pannese, E & Reale, E 1971 Histochemical localization of acetylcholinesterase activity in the inner ear *Acta Otolaryng* (Stockh ), Suppl *279*

Jasser, A & Guth, P S 1973 Synthesis of acetylcholine by the olivo-cochlear bundle *J Neurochem 20*, 45

Katsuki, Y 1966 Pharmacological investigations of cochlear responses and of olivo-cochlear inhibition *J Neurophysiol 29*, 94

Martini, D V 1940 Liberazione di sostanza acetilcolino simile nel-L'orecchio interno durante la stimolazione sonora *Boll Soc Ital Biol Sper 15*, 1102

McKinstry D N , Koelle, G B 1967 Effects of drugs on acetylcholine release from the cat superior cervical ganglion by carbachol and by preganglionic stimulation *J Pharm Exp Ther 157*, 328

Phillis, J W 1970 *The pharmacology of synapses* Pergamon Press, Oxford

Schuknecht, H F 1953 Techniques for study of cochlear function and pathology in experimental animals *Arch Otolaryng* (Chic ) *58*, 377

Sohmer, H , Feinmesser, M 1963 Studies on the influence of acetylcholine, eserine and atropine on cochlear potentials in guinea pig and the cat *Arch int Pharmacodyn 144* 446

Tanaka, Y, & Katsuki, Y , 1966 Pharmacological investigations of cochlear responses and of olivo-cochlear inhibition *J Neurophysiol 29*, 94

Tasaki, I & Fernandez, C 1952 Modification of cochlear microphonics and action potentials by KCl solution and by direct currents *J Neurophysiol 15*, 497

Werman, R 1966 Criteria for identification of a central nervous system transmitter *Comp Biochem Physiol 18*, 745

Whitfield, I C 1967 Discussion following a paper by J Fex In *Hearing mechanisms in vertebrates* (eds A V S Dereuck & J Knight), p 184 Little Brown & Co , Boston

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# GENETIC PROGRESSIVE HEARING LOSS IN THE C57/b16 MOUSE

*Relation of Behavioral Responses to Cochlear Anatomy*

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(Received July 7, 1973)

*Abstract* Mice (strain C57/b16) were studied using light microscopy The inner ear developed normally However, at the age of 3 months, degeneration began in the organ of Corti, beginning in the basal portion and progressing gradually to involve all of the cochlea By the end of one year, the organ of Corti had disappeared from the basal turn and persisted only in a degenerative form in the apical portion

Behaviorally, the hearing of this animal was near normal at the age of 2–3 months, except for the frequencies 30 Kc and above As the animal grew older, hearing gradually deteriorated and the high frequencies were totally lost the low frequencies were preserved, but at an elevated threshold

Interestingly enough, at the time when this animal's threshold of hearing above 30 Kc was higher than normal, the organ of Corti appeared normal Similarly, at the end of one year, light microscopy revealed an atrophic and disorganized organ of Corti present only in the apical turn of the cochlea (the rest of the cochlea having degenerated totally) and yet this animal maintained hearing for the low frequencies although at an elevated threshold It appears that light microscopy alone cannot discriminate between the functional and non functional state of the cochlea

The mouse is a particularly good animal for the study of auditory and vestibular systems There are several species which carry different genes for hereditary deafness and also genes for vestibular degeneration The auditory system has been most widely studied because of our interest in human hereditary deafness and also because of the modern advances in the fields of auditory neurophysiology and human communication The vestibular system in deaf mice has received only limited investigation

In the study of various sensori neural hearing losses, the aim of investigators has been to relate the behaviorial responses of the human to the anatomical findings of the inner ear The techniques used are based on the early work of Guild (Guild, 1919, 1932) Such material is now more readily available because of the establishment of human temporal bone bank centers Because of the inherent difficulties of studying the developmental and degenerative hearing losses, our investigators must turn again to animals and try to relate the behaviorial and anatomical findings Such studies are indeed fewer in mice because of the difficulties in the training of these animals to obtain the behavioral puretone thresholds for hearing Hearing in mice has often been tested by a Preyer reflex—a twitching of the pinna to a sudden tap on a metal pan Threshold hearing measurements have been made using such an approach (Powers et al, 1966) However, there are several obvious objections to using such methods In a study published by Birch et al (1968), a method was developed whereby absolute measurements for pure tone thresholds in the normal CBA-J mice were made using operant conditioning methods with positive reinforcement The behavioral

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thresholds were compared with physiological data (cochlear potentials, single units from the cochlear nucleus and GSR responses as reported in other studies) (Mikaelian, 1966, Mikaelian et al , 1965, Berlin et al , 1968) and were found to correlate fairly well in the range of frequencies from 10 to 30 Kc The thresholds were lowest in these ranges However, there was little correlation of results with frequencies above 30 and below 10 Kc

The C57/b16 strain of mice was selected for the present study because of the initial observations by Kocher (1960) that these mice hear and respond well in early life (first 2 to 3 months of life) and then gradually lose their hearing as measured by the Preyer reflex The clinical picture closely resembled certain types of hereditary hearing losses observed in humans The above observations prompted us to investigate this strain of mice in a strictly controlled experiment and study the developmental behavioral responses and relate the findings to the changes that occurred in the inner ear

## METHOD

The trained subjects were 17 C57/b16 mice bred from stock obtained from the Jackson Labora- [illegible]y, Bar Harbor, Maine They were studied in [illegible] replications (group I $N=5$, groups II and [illegible] $N=6$ each) extending over a 28 to 30 month period of time The apparatus and training procedures are described by Birch and co workers in full detail (1968) A brief description of the method of training is presented here Training was conducted in a Foringer cage with transparent plastic walls and a grate floor and ceiling An opaque plastic barrier under which the mouse could walk divided the cage in half Projecting into the cage from the right wall were a light metal lever and a blunt hypodermic needle, number 20 When the mouse pressed the lever a solenoid was activated so that a drop of sweetened water was automatically dispensed from the tip of the needle

All decibel readings for the pure tone are relative to 0 0002 dynes per centimeter square

Table I *dB SPL output at maximum intensity*

| Frequency | SPL at O Attenuation |
|---|---|
| 2 950 | 74 8 dB |
| 5 100 | 83 8 |
| 9 800 | 84 8 |
| 19 600 | 93 8 |
| 30 000 | 100 8 |
| 39 500 | 99 3 |
| 57 000 | 90 8 |
| 81 000 | 79 8 |
| 99 500 | 68 3 |

(SPL) The experimenter initiated the train o[f] tone pulses which was automatically timed t[o] last 10 sec by means of a clock timer During this tonal period, the mouse could cross to th[e] right of the cage, press the lever, and lick fro[m] the blunt needle Training began at 4 weeks o[f] age Each animal was deprived of water for 18 t[o] 20 hours before each session A 39 500 cycl[e] tone at 55 dB was used for training The anima[l] was conditioned to press the lever during tone and to withhold pressing during silence

When the animals were pressing the lever on a[t] least 80 % of all tonal intervals threshold testin[g] was begun Two or three tones were tested during each daily session There were nine tones in all (2 950, 5 100, 9 800, 19 600, 30 000, 59 500, 57 000, 81 000, and 99 500 cycles per sec) The method of limits descending series was used The most intense tone was 55 to 60 dB SPL If no response was made at that intensity the attenuation was decreased by 10 dB until a response occurred or until the maximum intensity was reached Table I shows the maximum SPL possible for each tonal frequency as measured by a Bruel & Kjaer ½ inch probe microphone near the position of the mouse at the start of each trial The values of the decibel readings are based on two calibration measurements the microphone being placed at two different positions near where the mouse would be at the start of the training Tones were presented in random order The threshold was defined as 5 dB lower than the least intense sound to which the mouse responded Threshold testing was continued to 12 months for 6 animals, 16 months for 5 mice and up to 21 months for 6 mice

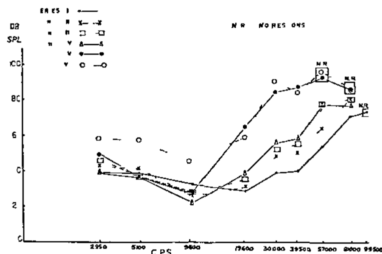



*Fig 1* Pure tone threshold changes with age C57/b16 mouse

The threshold SPL for each tone is based on the mean five different measurements It required 2 to 4 weeks in general to test a complete series of tones five times each The criterion for eliminating a tone from the testing series was that no response (NR) occurred on four out of the five consecutive measures

*Histological studies*

A series of C57/b16 mice were studied histologically (light microscopy) at various ages The animals were anesthetized with intra peritoneal injection of 0 2 cc of a 20 % solution of urethane and perfused with normal saline through the left ventricle followed by 10 % formalin (Lilly s neutral) The heads were removed trimmed and decalcified and then embedded in celloidin

They were then sectioned serially at 10 $\mu$m and every fifth section was stained with hematoxylin and eosin and mounted for study

## RESULTS

*A Behavioral*

Fig 1 shows the changes in pure tone sensitivity with age Table II shows the threshold values and standard deviations Thresholds for series I IV are computed by assigning to each mouse the mean point to the nearest ¼-month between his age at the beginning of the five measures and

Table II

T = Threshold
S D Standard dev at on

| Frequency (cps) | Age in months | | | | | | | | | | | | Normal CBA mouse | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 2 28 | | 3 ?1 | | 3 93 | | 4 86 | | 10 74 | | 17 22 | | | |
| | T | S D | T | S D | T | S D | T | S D | T | S D | T | S D | T | S D |
| 2 950 | 40 | 12 8 | 44 | 7 9 | 44 | 7 5 | 40 | 7 2 | 49 | 7 0 | 59 | 12.2 | 40 | 15 3 |
| 5 100 | 38 4 | 10 9 | 41 | 12 3 | 36 | 5 8 | 35 | 9 5 | 38 | 7 00 | 58 | 19 ? | 38 | 14 3 |
| 9 800 | 3? | 12 | 27 | 7 3 | 29 | 13 2 | 23 | 8 00 | 29 | 9 00 | 45 | 16 8 | 29 | 20 5 |
| 19 600 | 28 | 11 2 | 3? | 8 5 | 36 | 10 2 | 38 | 6 00 | 66 | 11 1 | 59 | 13 4 | 30 | 20 0 |
| 30 000 | 39 | 10 6 | 48 | 10 6 | 51 | 11 7 | 53 | 9 4 | 84 | 12 2 | 90 | 14 1 | ?8 | 18 6 |
| 39 500 | 40 | 10 1 | 50 | 13 6 | 55 | 10 8 | 58 | 8 00 | 88 | 15 1 | 84 | 20 00 | 38 | 17 |
| 57 000 | 54 | 11 9 | 63 | 10 2 | 80 | 10 4 | 81 | 11 6 | | N R | | N R | 36 | 20 2 |
| 81 000 | 71 | 5 5 | 78 | 5 29 | | N R | | N R | | N R | | N R | ?0 | 12 1 |
| 99 500 | | N R | | N R | | N R | | N R | | N R | | N R | | N R |

Table III *Mean and age range of the mice*

| Series | Mean age (months) | Range (months) |
|---|---|---|
| Series I | 2 28 | 1 75–3 00 |
| Series II | 3.21 | 2 25–4 00 |
| Series III | 3 95 | 2 75–5 00 |
| Series IV | 4 86 | 3 75–7 25 |
| Series V | 10 74 | 9 00–13 00 |
| Series VI | 17 22 | 14 50–21 50 |

his age at the end These mean points were then averaged for all 17 mice (Table III) The mean and age range for each series are given in this table

The results show a decided progressive high tone loss with age Sensitivity to tones 9 800 Hz and below show *no loss up to the mean age of* 10 74 months By 17 22 months, the losses are between 10 and 20 dB for these tones The loss is gradual For frequencies 19 600 cycles and above all show decreased sensitivity between the first and second series, between the second and third series, and between the third and fourth series (except for 57 000 cycles which was the same in the third and fourth series) By 10 months there was no response at 57 000 cycles and above

Birch and co-workers (1968) published pure tone thresholds for normal CBA-J mice using he same apparatus and training procedures *g 2 contains a comparison between the CBA-J* ce and the series I and Series VI thresholds of the mice of the present study The age of the normal CBA-J mice at the initial measurement was 4 to 7 months The Series I threshold curve diverged at 30 000 cycles and above, indicating that the very high frequency sensitivity of the C57/b16 mice is inferior to that of the normal CBA-J mice at an early age

## B *Histological studies*

Two mice of the C57/b16 strain at various ages were sacrificed for histological study The ages at sacrifice were 1 week, 2 weeks, 3 weeks, 4 weeks, 2 months, 3 months, 5 months, 12-14 months, 18 months, and 19-20 months

A total of 18 C57/b16 mice were sacrificed A controlled series of normal CBA-J mice was sacrificed at comparable ages No degenerative *changes were seen in the inner ears up until the* age of 3 months Until then the organ of Corti had a normal structure

The earliest change seen in the C57/b16 mi was observed at the age of 3 months (Fig 3 This was apparent first in the basal turn and varied in extent In some, a disorganized orga of Corti was seen where some of the hair cel were missing, the supporting cells and the pilla cells were clumped together All structure had a atrophic appearance Tunnel fibers could still b seen The stria vascularis was normal, and th ganglion cells were well preserved and had a nor *mal population In the other turns,* the organ o Corti appeared structurally normal

In the fourth and fifth months (Fig 4), th

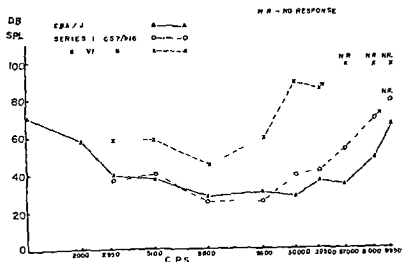



*Fig 2* Comparison of pure tone thresholds between the CBA J and the Series I and Series VI of the C57/b16 mice

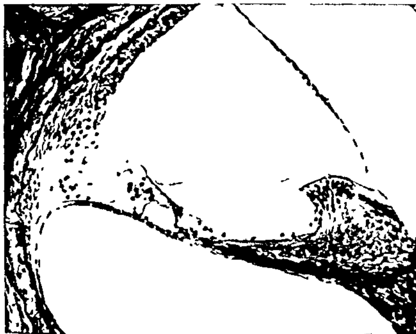


*Fig 3* Earliest changes seen in the organ of Corti C57/b16 mouse, age 3 months Animal no 3329, × 200 See text for description

degeneration involved both turns of the cochlea with complete absence of the organ of corti in the basal part However, in the uppermost areas the organ of Corti was somewhat disorganized—fewer hair cells were found and atrophic changes could be seen in both the hair cells and supporting cells The stria vascularis revealed some atrophy, which was present mostly in the basal area At this stage, also, the ganglion cells in the basal portion were fewer and showed atrophy

About the age of one year, the picture was almost uniform (Figs 5 and 6) Absence of the organ of Corti with atrophic stria vascularis, very few neurons in the modiolus in the region



*Fig 4* C57/b16 mouse, age 5 months Animal no 3305, × 75 See text for descriptions



*Fig 5* C57/b16 mouse 1 year old An mal no 3037 ×75 See text for descriptions

of the basal turn The apical turn showed a dis organized organ of Corti with atrophic hair cells and supporting cells (Fig 7) The neuron population was still preserved but slightly reduced

## DISCUSSION

The purpose of the animal investigation was to relate the degenerative changes of the Corti occurring in the C57/b16 maturing mouse with



*Fig 6* C57/b16 mouse Same as Fig 5 High power view 200 Basal turn



*Fig 7* C57/b16 mouse Same as Fig. 6, × 150 Apical turn See text for description

hearing loss determined by pure tone thresholds Deterioration of hearing began with the high frequencies and gradually involved the lower ones, but the animals never became totally deaf Considered anatomically, degeneration in the organ of Corti became apparent first in the basal portion of the cochlea at the age of 3 months, and gradually extended to the apex The pattern of degeneration of the organ of Corti consisted of disorganization of the organ of Corti and the hair cells, with atrophy setting in, and gradually the organ of Corti completely disappearing The spiral ganglion cells diminished progressively In the apical area of the cochlea the organ of Corti persisted in a dispersed manner, but one could still identify various atrophic hair cells and supporting cells The spiral ganglion cells were maintained in the upper part The stria vascularis became smaller and atrophic in the areas where the organ of Corti had completely degenerated The cochlear duct and scala media were preserved normally throughout The vestibular part of the labyrinth persisted normally

For the behavioral results the C57/b16 mice did not appear to possess normal thresholds for hearing in the frequencies of 30 000 cycles and above, as compared with the CBA-J mice (presumed to be normally hearing mice) The discrepancy was observed even before degenerative changes could be observed in the cochlea The picture resembled the previously reported observations in the Shaker-1 mouse, where this animal had lost hearing totally by the 22nd day after birth, and yet the organ of Corti appeared absolutely normal (Mikaelian & Ruben, 1964) On the other hand the low frequencies still persisted in the C57/b16 mice, though with an elevated threshold The organ of Corti in the apical portion of the cochlea appeared degenerated, yet this animal maintained hearing It must be that the remaining hair cells of the organ of Corti were still functioning in this area Of interest would be electronmicroscopic studies of the hair cells in this region and to try to determine the nature of innervation of these cells It is of great interest that such a disorganized organ of Corti was still functioning, as shown by the behavioral pure tone thresholds

If one were to make a general statement as to the pattern of degeneration, the direction of the affection was from base to apex, and t



*Fig 5* C57/b16 mouse 1 year old Animal no 3037 ×75 See text for descriptions

of the basal turn The apical turn showed a disorganized organ of Corti with atrophic hair cells and supporting cells (Fig 7) The neuron population was still preserved but slightly reduced

## DISCUSSION

The purpose of the animal investigation was to relate the degenerative changes of the Corti occurring in the C57/b16 maturing mouse with



*Fig 6* C57/b16 mouse Same as Fig 5 High power view ×200 Basal turn

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# THE EFFECT OF PRESERVATION ON THE BEHAVIOUR OF HOMOLOGOUS OSSICULAR GRAFTS

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(Received August 6, 1973)

*Abstract* The present study deals with the effect of several preservation methods on the behaviour of incus grafts in rats after orthotopic and heterotopic transplantations The following conclusions can be drawn 1 Preservation seems to influence both the potential for new bone formation and the antigenic properties of an ossicular graft 2 Preservation in cialit and alcohol and freeze drying preserves the osteogenic properties to a variable extent 3 Bone formation is absent after boiling and after preservation in formaldehyde 4 The antigenic properties are affected by storage in alcohol and cialit and by boiling 5 Preservation of the antigenic properties of the graft does occur after freezing and freeze drying and after storage in formaldehyde 6 Typical reaction of transplantation and resorption of the graft are only observed after heterotopic transplantation in muscle

From clinical experience we know that ossicular transplants for tympanoplasty are well tolerated in the middle ear There are no definite reports indicating that these transplants are extruded or rejected like true organ transplants in other parts of the body However, the lack of understanding of the fundamental reasons underlying this, demands a critical attitude towards their use Many basic biological problems remain to be solved Of these most important ones are the problems concerning the possible antigenicity of these grafts and the problems of revitalisation and resorption

From experiments in animals it has become clear that reactions against either fresh or preserved homologous grafts do not seem to occur in the middle-ear or at least are so weakened that they cannot be traced by the methods used up to date When a fresh homologous incus is transplanted into muscle signs of a transplantation reaction become visible which shows the antigenicity of such a graft (Van den Broek, 1968, Kuijpers & Van den Broek, 1972) and the question arises whether in the long run any inferior behaviour in the middle ear could result from the preservation of these antigens Experiments by Kastenbauer (1972) have learned that the antigens from an ossicular graft can elicit a second set reaction in the middle-ear of previously sensitised animals

A closely related problem is the influence of preservation on the revitalisation of the graft From the literature we know that there is no consensus of opinion on the degree of bone formation in ossicular grafts (Wilson et al, 1966, Smith & Overton, 1968, Benitez & McIntire, 1968, Dawkins, 1971, Kerr & Smyth, 1971)

The aim of this study has been to study the effect of different preservation methods on both the antigenic properties of an ossicular graft and on the potential for the formation of new bone Because boiling of an ossicle denatures all the proteins in a graft and, as shown in previous experiments, abolishes the potential for the formation of new bone, this method is not included

Both orthotopic and heterotopic transplantations were performed Heterotopic transplantation in leg muscle has the advantage of a more intimate contact between graft and host tissue which will result in more pronounced effects than in the middle-ear

Table I *Summary of the experiments performed*

| Preservation method | Preservation time | No of rats |
|---|---|---|
| Alcohol 70 % | 1 week | 14 |
| | 9 weeks | 9 |
| | 19 weeks | 4 |
| Formalin 4 % | 1 week | 10 |
| | 8 weeks | 8 |
| | 17 weeks | 8 |
| Cialit | 1 week | 8 |
| | 2 weeks | 5 |
| | 7 weeks | 8 |
| | 20 weeks | 7 |
| Freezing | 33 weeks | 4 |
| Freeze drying | 2 weeks | 10 |

## MATERIALS AND METHODS

Homologous transplantations of incudes were performed between the middle ears of two genetically different strains of young adult rats The incudes were removed from one strain, preserved in different ways for varying periods of time, after which they were transplanted into the middle ear of the second strain At the same time heterotopic implantation of incudes preserved in the same way was performed in muscle All grafts were rinsed in saline before introduction into the middle ear and muscle, except those preserved by freeze drying

Table I shows a schematic representation of the different types of preservation used as well as the preservation time and the number of experiments performed After varying survival times the animals were sacrificed, the middle ears and muscle fragments containing the grafts removed and processed for microscopical study as described previously (Van den Broek & Kuijpers, 1967) From the serial sections the most important features (overall vitality, formation of bone reaction of the recipient site, and the degree of resorption) were recorded and tabulated

### *Alcohol*

Alcohol 70 % has been used by many authors as a preservative for ossicles Its biological effect is dehydration, which results in coagulation of the proteins, while the acidic or basic character of the proteins remains unaltered Its antiseptic qualities have been known for a long time and add to its usefulness as a preservative for grafts

In Table II the results of this group of experiments are recorded The local reactions towards an alcohol preserved implant are minimal The

Table II *Preservation in alcohol*
Res = resorption Ot = otitis media

| | 1 week | | | | 9 weeks | | | | 19 weeks | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Ear | | Leg | | Ear | | Leg | | Ear | | Leg | |
| Survival time | New bone | Res | New bone | Res | New bone | Res | New bone | Res | New bone | Res | New bone | Res |
| 10 days | – | | – | | – | – | | + | | | – | – |
| 10 days | | | – | | – | – | | + | | | | |
| 3 weeks | | | – | + | – | – | | + + | | – | | – |
| 4 weeks | | | | | – | – | | + + | | | | |
| 4 weeks | | | | + | | | | | | | | |
| 4 weeks | | | + | + | | | | | | | | |
| 4 weeks | | | | + + | | | | | | | | |
| 4 weeks | | | | + + | | | | | | | | |
| 3 months | | – | | + | | | | | | | | |
| 3 months | | – | | + + | | | | | | | | |
| 3 months | | – | | + + | | | | | | | | |
| 3 months | | – | + | + | | | | | | | | |
| 4 months | + | | + | + | – | – | | | | | | + |
| 4 months | | | | + | – | – | + | + + | | | | |
| 12 months | | | | | ± | | | + + | | | | |
| 12 months | | | | | + | Ot | | + + | | | | |
| 12 months | | | | | + | – | + | + + | – | | + | + + |



*Fig 1* Cialit preserved (7 weeks) incus in muscle after 18 months Note small areas of new bone formation (←)

vitality of the implant is entirely lost after the storage in alcohol and during the first weeks the incus appears entirely dead Formation of new bone is first found after 4 weeks From 3 months onwards replacement by new bone is more marked especially in the heterotopic grafts, which in nearly all cases showed signs of osteoneogenesis In the middle-ear only 7 grafts were found with signs of bone formation This bone formation, however, has been rather scanty There was no relation between the time of storage and the degree of osteogenesis appearing in these grafts Even after storage for 19 weeks, bone formation was observed Although resorption was noted in muscle, this was never found in the middle ear

*Cialit*

Cialit is an organic mercury compound with bactericidal and fungicidal properties It has been used since 1954 in orthopaedics for preservation of bone and was first used in otology by Marquet (1966) as a preservative of homograft drum membranes For these purposes a solution of 1 5000 in distilled water is used

In Table III a survey of the experiments is recorded together with the most important findings regarding bone formation and resorption It can be seen that also after this type of preservation new bone can be found in the incus, which is also slightly more pronounced after heterotopic transplantation The reaction of the recipient site at 10 days consists of a minimal cellular infiltration which has disappeared after a fortnight and compares very well with the reaction around the alcohol preserved grafts

The formation of new bone in these grafts was generally restricted to small areas (Fig 1) In one of the specimens the narrow spaces looked wider than normal, otherwise no signs of resorption were found in the middle-ear In muscle, moderate resorptive activity was found No significant differences between alcohol- and cialit-preserved grafts became apparent and the behaviour of ossicular grafts after preservation and in cialit or alcohol seems to be very similar

*Formaldehyde*

Incus grafts were preserved for 1, 8 and 17 weeks in formaldehyde, 4 % solution, buffered at pH 7. In contrast to alcohol, formaldehyde is a non-coagulant fixative and many of the hydrophilic groups retain their relation with water molecules

The histologic findings are summarized in Table IV It can be seen that in none of these grafts was formation of any new bone found. One of the most remarkable findings of these experiments has been the persistence of the donor

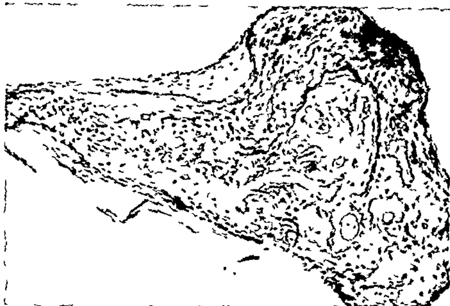


*Fig. 2.* Formaldehyde preserved (8 weeks) incus in the middle ear after 4 months. Most osteocyte nuclei are still present.

osteocyte nuclei in these formalin fixed grafts even after prolonged time in the recipient (Fig. 2). With all the other preservation methods, total disappearance of the donor osteocyte nuclei has occurred within a fortnight.

In the middle-ear no reaction to the grafted ossicle was present. In muscle, quite extensive resorption was apparent after 4 months, illustrated by the presence of a considerable number of osteoclasts. Furthermore, collections of mononuclear cells have been seen around these grafts, starting with the shortest survivals and persisting, although to a lesser extent, throughout the observation period (Fig. 3). These findings are highly suggestive of transplantation phenomena as observed when fresh homologous grafts are used (Kuijpers & Van den Broek, 1972). The absence of this reaction in the middle-ear is also in agreement with earlier findings when fresh homografts failed to reveal any reaction to transplantation in the middle-ear as opposed to fresh grafts in muscle.

In order to exclude any possible role of formaldehyde as an aspecific stimulus causing a mono-



*Fig. 3.* Formaldehyde-preserved (8 weeks) incus in muscle after 4 months. Extensive monocellular reaction is clearly visible.

Table III *Preservation in cialit*

Res resorption Ot otitis media

| | 1 week | | | | 2 weeks | | | | 7 weeks | | | | 20 weeks | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Ear | | Leg | | Ear | | Leg | | Ear | | Leg | | Ear | | Leg | |
| Survival time | New bone | Res | New bone | Res | New bone | Res | New bone | Res | New bone | Res | New bone | Res | New bone | Res. | New bone | Res. |
| 10 days | | − | | ± | − | | − | | | | | | | | | |
| 10 days | | | | + | | − | | ∓ | | | | | − | | | |
| 2 weeks | | | − | + | − | | − | | | | − | | | | | |
| 4 months | − | | | + | | − | | − | | | | − | | | | |
| 4 months | | | | + | | | | | | | | ± | − | Ot | | |
| 4 months | − | | − | + | | | | | | | | | − | − | | |
| 4 months | ± | | + | + | | | | | | | | | | | − | − |
| 4 months | ± | − | + | + | + | | + | | | | | | | | | |
| 7 months | − | | | − | | | | | ± | | − − | | | | | |
| 12 months | | − | | + | | | | | | | | | | | | |
| 12 months | + | | + | + + | | | | | | | | | | | − − | + |
| 18 months | | | | | | | | | + | − | | | | | | |
| 18 months | | | | | | | | | | | + | | | | | |

cellular reaction, additional experiments were performed by first boiling the incudes and subsequently storing these in formaldehyde for 1 week No monocellular reaction was found around these incudes after about 3 months

*Storage at low temperature*

Another method of preservation is storage at temperatures below 0°C Two different methods have been used in these experiments By the first the incudes were put into distilled water immediately after removal frozen to −25°C and then stored at the same temperature (freezing) By the second method these incudes were frozen immediately to −85°C in solid carbon dioxide and consequently lyophilised at 30°C and stored for 2 weeks at the same temperature (freeze-drying) Because the storage time in both groups was very different (33 *v* 2 weeks) the results are only partly comparable

These two groups of experiments are summarised in Table V No bone formation was found

Table IV *Preservation in formaldehyde*

Res = resorption React = reaction consisting of mononuclear cells

| | 1 week | | | | | | 8 weeks | | | | | | 17 weeks | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Ear | | | Leg | | | Ear | | | Leg | | | Ear | | | Leg |
| Survival time | New bone | Res | React | New bone | Res | React | New bone | Res | React | New bone | Res | React | New bone | Res. | React | |
| 2 weeks | | | | − | + | + + | | | | | | + | − | | | |
| 2 weeks | | | | | | + + | | | | | | | | | | |
| 2 weeks | | | | | | | | | | | | | | | | |
| 4 weeks | | | | | | | | | − | | + | + + | | | | |
| 4 months | | | | | | + | | | − | − | + | + | − | | | |
| 4 months | | | | | | | | | | − | + + | + | | | | |
| 4 months | | | − | | | + | | | | | + | + + | | | | |
| 13 months | | | | | + | ± | | | | | | − | − | | | |
| 13 months | | | | | | ± | − | | | − | + + | − | | | | |
| 13 months | | − | | | | ± | | | | | + | − | | | | |

Table V *Preservation by freeze drying and by freezing*

Res = resorption React = reaction consisting of mononuclear cells Ot = otitis media

| | Freeze drying | | | | | | Freezing | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Ear | | | Leg | | | Ear | | | Leg | | |
| Survival time | New bone | Res | React | New bone | Res | React | New bone | Res | React | New bone | Res | React |
| 2 weeks | – | – | – | – | – | – | – | – | Ot | – | ++ | + |
| 2 weeks | – | | Ot | ± | ± | ± | | | | | | |
| 4 weeks | ± | – | – | – | ++ | ++ | | | | | | |
| 4 weeks | ± | | – | – | ++ | ++ | | | | | | |
| 4 months | + | – | – | + | ++ | + | – | – | – | + | + | + |
| 4 months | + | – | – | – | – | – | – | – | – | – | + | ± |
| 9 months | ++ | – | – | – | + | ± | | | | | | |
| 14 months | + | – | Ot | – | + | ± | – | – | – | – | ++ | + |
| 14 months | – | – | | – | ++ | + | | | | | | |
| 14 months | – | – | – | + | ++ | ± | | | | | | |

in incudes in the middle ear when these were frozen In muscle in only one of the incudes was bone formation observed Incudes which were freeze dried and kept for 2 weeks were found to show bone formation in most cases after 4 weeks (Fig 4) In muscle the result was less prominent and bone formation was found in only three of the specimens As to the reaction around these grafts one can see that in both groups marked monocellular reactions were present to an extent not observed around any of the other grafts except in those which were stored in formaldehyde (Fig 5) It is reasonable to assume that this monocellular reaction also depends on the preservation of antigenic properties when this method of storage is used

*Decalcification*

Decalcification of both fresh incudes and incudes stored for 2 days in alcohol or formaldehyde was performed in EDTA (10 % pH 7 0) for 10 days at 4°C Subsequently the incus was transplanted At histological examination the incudes were found to be infiltrated by connective tissue cells (Fig 6) No difference could be observed between the behaviour of the fresh and the pre

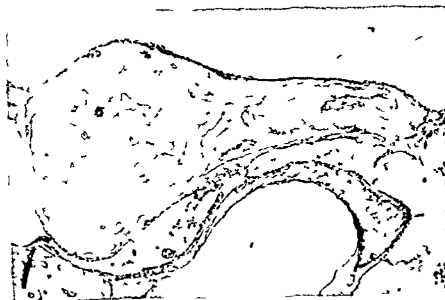


*Fig 4* Freeze-dried incus in the middle ear after 9 months Area of new bone in the long process of the incus



*Fig 5* Freeze-dr ed incus in muscle after 4 weeks Note the monocellular react on present around the t p of the ncus

served grafts The infiltration by connective tis sue cells was much more pronounced in muscle than in the middle ear It was quite remarkable that hardly any osteoclasts were observed in the incudes in muscle even after longer survival times which contrasts markedly with all the other experiments performed Often fat was found surround ng the incus in the muscle and somet mes replacing part of the graft In the middle-ear no resorptive activity was found up to one year

## DISCUSSION

The findings of the present study indicate that the behaviour of a grafted ossicle both in the middle ear and in muscle is related to the mode of preservation

Two properties of the graft seem to be influ enced by the preservation technique First the potential for remodelling by new bone and sec ond the antigenic action of the graft These processes are at least partly independent



*F g 6* Incus decalcif ed w th EDTA transplan ed nto the m ddle ear Results after 1? months Incudo-mallear jo nt

From the results obtained it appears that storage in alcohol, cialit and freeze drying (and presumably also freezing) preserves the potential for new bone formation However, this new bone formation mainly confined to the central parts of the grafts, starts much later than in fresh auto- and homografts In the latter cases this new bone visible within a few days after transplantation, is probably formed by surviving periosteal cells of the graft (Van den Broek & Kuijpers, 1967)

When the ossicles are stored in formaldehyde or denatured by boiling or drying as previously reported (Van den Broek, 1968) no bone formation has been found, even after prolonged presence in the middle-ear or in muscle

From the available data it follows that new bone formation in preserved ossicles is dependent on host cells, which are probably transformed into osteogenic cells under influence of the graft No conclusion can be drawn from the character of the substances in the graft which are responsible for this transformation process The preservation methods after which new bone formation was found have in common that the original molecular structure of the tissue components is more or less retained, although after preservation in formaldehyde which also preserves the molecular structure very well, bone formation was absent

It is noteworthy that careful decalcification at neutral pH with EDTA appeared to destroy this potential in alcohol-preserved specimens In addition the decalcification seems to change the structure of the organic matrix in such a way that connective cells can easily penetrate into the graft between the fibres

The second problem which appears to be related to the preservation method is the antigenicity of the graft It seems that the antigenic properties of the graft are preserved after storage in formaldehyde, freeze drying and freezing, but not after storage in alcohol or cialit, or after boiling, as far as could be detected by the methods used This was concluded from the presence of a characteristic monocellular reaction around the grafts implanted in muscle similar to that observed previously around fresh homologous grafts in this location (Kuijpers & Van den Broek, 1972) These findings do suggest that those preservation methods which do not alter profoundly the nature of the proteins, do preserve at least some of the antigenic properties The absence of a monocellular reaction towards these grafts in the middle-ear confirms the previously stated hypothesis that the middle ear reacts differently and that these processes, if present, are very much mitigated or retarded Related to this problem, experiments have been performed by Kastenbauer (1972), who observed reactions suggestive of a transplantation reaction in the middle-ear only after previous sensitation (second set phenomenon), which might mean that transplantation immunity in the middle-ear is not at all to be ignored Hitherto, however there is no clear evidence that the clinical results of using preservation methods which, according to our experiments, seem to preserve antigenic properties are any inferior (Perkins, 1971), although the inferior results reported by Smyth (1972) with freeze-dried homograft drums might be significant

In conclusion, there is no doubt that the behaviour of a dead preserved ossicle is a dynamic one, the ultimate fate of which is difficult to predict Therefore, in using these ossicles, one should take in to account the above-mentioned effects of the various preservation methods

## ZUSAMMENFASSUNG

Das Verhalten konservierter homologer Amboss-Transplantate nach orthotopischer und heterotopischer Transplantation wird im Tierexperiment untersucht Zusammenfassend könnte folgendes festgestellt werden 1 Konservierung beeinflusst die osteogenetische Potenz und die Antigenität der Transplantate 2 Aufbewahrung in Cialit und Alkohol und Einfrieren und Lyophilisieren konserviert in verschiedenem Masse die osteogenetische Potenz 3 Diese Potenz wird völlig destruiert durch Kochen und Aufbewahren in Formalin 4 Die Antigenität wird durch Kochen und Aufbewahrung in Alkohol und Cialit b•seitigt 5 Einfrieren Lyophilisieren und Konservieren mit Formalin bewahrt mindestens ein Teil der Antigenität der Amboss Transplantate 6 Abwehrreaktionen gegen homologe Amboss Transplantate werden nur beobachtet nach heterotopischer Transplantationen in Muskeln

## REFERENCES


Benitez, I T & McIntire, C1 1968 Incus homografts viability and rate of bone repair by tetracycline labels and fluorescent microscopy in the cat *J Laryng 82*, 23

Dawkins R S 1971 Experimental middle-ear homografts *J Laryng 85*, 315

Kastenbauer, E. R 1972 Tierexperimentelle Untersuchungen uber das immunologische Verhalten allogener Gehorknochelchen Transplantate *Arch Klin Exp Ohr Nas Kehlkopfheilk 201*, 332

Kerr, A G & Smyth, G D L 1971 The fate of transplanted ossicles *J Laryng 85*, 337

Kuijpers W & Van den Broek, P 1972 Fundamental aspects of incus transplantation. *Laryngoscope 82*, 2174

Marquet, J F E 1966 Reconstructive microsurgery of the eardrum by means of a tympanic membrane homograft *Acta Otolaryng* (Stockh ) *62*, 459

Perkins, R 1971 Homograft tympanic membrane with suture sling *Laryngoscope 80*, 1100

Smith, C W & Overton S D 1968 The fate of middle-ear ossicle grafts *Laryngoscope 78*, 1002

Smyth, G D L & Kerr, A G 1969 Tympanic membrane homografts *J Laryng 83*, 1061

Smyth, G D L 1972

Touma, J B & Maguda, Th A 1973 Ossicular chain transplantation in cats *Ann Otol 82*, 62

Van den Broek, P 1968 The fate of incus grafts *Thesis*, Nijmegen

Van den Broek, P & Kuijpers, W 1967 Incus autografts and homografts in rats *Arch Otolaryng* (Chic ) *81*, 287

Wilson, D F , Pulec, J L & Vliet P D van 1966 Incus homografts in cats *Arch Otolaryng* (Chic ) *83*, 554



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# OTITIS EXTERNA AND ALLERGIC CONTACT DERMATITIS

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*Abstract* Among 98 patients with otitis externa 34 (35 %) gave positive response to patch tests The most common finding was allergy to drugs, as a rule neomycin On the basis of the study it is concluded that the incidence of allergic contact dermatitis is 12–22 % among patients with otitis externa

Otitis externa designates a number of different disease conditions of the auditory canal of uncertain aetiology and pathogenesis The disease is of great practical importance Singer & Freeman, in 1952, found that the Pseudomonas group was the most important aetiological agent According to their investigations fungi played a limited aetiological role In Jones' (1971) opinion e primary factor is the development of allergy bacteria and fungi Baumann & Carr (1961) ere able to disprove the theory that cerumen from normal ears possessed antibacterial properties as compared with cerumen from ears affected with otitis externa

The aetiology is presumably multifactorial Local factors, such as temperature and humidity, are important Infection is probably in most cases secondary The topical use of antibiotics may convert an "idiopathic" eczema into allergic contact dermatitis Lastly, the aetiology may be primary contact dermatitis McKelvie & McKelvie (1966) found 5 cases of allergy in a study of 113 patients, but their patients were tested only for chrome, nickel, and neomycin

The present study was designed to elucidate the incidence of primary and secondary allergic contact dermatitis in patients with otitis externa

## METHOD AND MATERIAL

The material comprises 100 consecutive patients referred to the E N T Department by otological practitioners To avoid a preponderance of particularly severe cases, the referring otologists were requested to refer all their cases of otitis externa to us during the period concerned No selection was made

The history-taking and physical examination were carried out and recorded according to a fixed plan The diagnosis of otitis externa was based on the patients' symptoms in the form of itching discharge, and pain and on the objective findings Thereafter, swabs were taken from the auditory canal The Department of Dermatology carried out an examination for fungi, standard eczema tests, patch tests with sulphathiazole cream, $HgCl_2$, shellac, Furacin® iodoform Otoafanal (procaine chloride), Ciloprin® (4-carboxymethylamino-4-aminodiphenylsulfone) and patch tests using the drugs that the patient has had for treating the otitis externa Finally, patch tests of the patient's cosmetics were applied The patients attended again for reading of the patch tests No therapeutic trials or follow-up were done

Of the 100 patients 62 were females and 38 males Two patients failed to appear after the patch tests had been applied and were therefore excluded Two thirds of the patients had been suffering from their disorder for more than 2 months About two-thirds had previously had

Table I *Distribution of the 57 cutaneous allergies found in 34 patients with otitis externa The allergy was demonstrated by positive patch test*

| Substance | Number |
|---|---|
| 1 *Drugs* | *30* |
| Neomycin sulphate | 8 |
| Coal tar | 4 |
| Merthiolate® (preservative *int al* in Synalar®) | 3 |
| Wood tar | 3 |
| Mercuric chloride | 2 |
| N trofurazone (Furacin®) | 2 |
| 4-carboxymethylamino-4-aminodiphenyl sulfone (Ciloprin®) | 1 |
| Chloramphenicol | 1 |
| Chlorquinaldol (Sterosan®) | 1 |
| Dequalonium chloride (Danical®) | 1 |
| Paraben (preservative) | 1 |
| Polymyxin sulphate | 1 |
| Procaine chloride (Oto-afanal®) | 1 |
| Iodochlorhydroxyquin (Vioform®) | 1 |
| 2 *Chemicals* | *12* |
| Formaldehyde | 2 |
| Shampoo and hair dressings | 2 |
| Aminoazobenzene | 1 |
| Gum | 1 |
| Colophonium | 1 |
| Adhesive plaster | 1 |
| Paraphenylenediamine | 1 |
| Balsam of Peru | 1 |
| Turpentine | 1 |
| Hearing aid inserts | 1 |
| 3 *Metals* | *10* |
| Cobalt chloride | 4 |
| Nickel sulphate | 4 |
| Potassium bichromate | 2 |
| 4 *Miscellaneous* | 5 |
| Chrysanthemum | 2 |
| Primrose | 2 |
| Orange | 1 |

otitis externa The majority of the patients had been treated by their own otologist, many of them with little effect

## RESULTS

In 34 out of 98 patients with otitis externa one or more cutaneous allergies were found (35 %) Both sexes were equally represented A total of 57 allergies were found in these 34 patients Table I shows the distribution of the positive patch tests

The largest group is made up of drugs It will be seen that a risk of sensitization is involved by nearly all agents for topical application into the auditory canal Like others before us, we also found neomycin allergy to be common Otherwise, it is not possible to deduce from the tabulation which agents carry the greatest risk of sensitization The second largest group consists of various chemicals Paraphenylene diamine is used *inter alia* in hair dyes The metal allergies are of interest to the otologist in connection with irrigation by metal syringes after which iatrogenic otitis externa has been observed and in the use of hairpins and matches for scratching the ear

Table II *Patients' assessment of the effect of treatment given*

$n = 34$ in the allergy group and $n = 64$ in the non allergy group $p < 0.05$

| | Allergy | No allergy |
|---|---|---|
| No effect | 85 % (28) | 67 % (37) |
| Good effect | 15 % (5) | 23 % (18) |
| Not treated | (1) | (9) |

In an effort to demonstrate differences between the allergic group and the patients in whom no allergy was found, the following factors were investigated

1 Effect of the treatment given
2 Site of the eczema
3 Duration of the disease
4 Symptoms
5 Tendency to relapse
6 Bacteriological findings

Table III *Site of lesions in the two groups The allergic contact dermatitis is more apt to spread on the auricle*

$p < 0.05$ $n = 34$ and $n = 64$

| | Allergy | No allergy |
|---|---|---|
| Auditory canal only | 48 % (16) | 68 % (43) |
| Auditory canal and auricle (in some cases retroauric ) | 52 % (17) | 32 % (20) |
| Unknown | (1) | (1) |

Table IV *Distribution of symptoms in the two groups There is no difference between the non-allergic and the allergic group with respect to the subjective symptoms*

($n = 34$ and $n = 64$)

| | Allergy | No allergy |
|---|---|---|
| Itching | 100 % (34) | 92 % (59) |
| Discharge | 71 % (26) | 75 % (48) |
| Pain | 42 % (16) | 39 % (25) |

Table II presents the patients' own assessment of the treatment given Among the patients with diagnosed allergy 28 (85 %) reported no effect, in the non-allergic group 37 (67 %) The difference is fairly significant ($p < 0.05$)

With respect to the site, Table III shows that allergic contact dermatitis is more apt than non-allergic eczema to spread on the auricle ($p < 0.05$)

It is not possible to decide, on the basis of the patients' symptoms, whether the condition is possibly interpretable as a development of allergy Table IV shows no difference between the two groups

There was no significant difference between the two groups with respect to duration or tendency to recurrence (*Tables V and VI*)

Concerning the bacteriological findings (Table VII), one might have expected to find Pseudomonas more often in the non-allergic group On the other hand, an allergic contact dermatitis might get infected, and a large number of the patients had been or were being treated

Lastly, pityriasis simplex was demonstrated in 10 patients, all of the group without demonstrable allergy

Table V *Duration of the disease in the two groups*

($n = 34$ and $n$ 64)

| | Allergy | No allergy |
|---|---|---|
| More than 2 months | 76 % (26) | 66 % (42) |
| Less than 2 months | 24 % (8) | 34 % (22) |

Table VI *Number of patients with a history of previous otitis externa There was no greater tendency to relapse in the allergic group*

($n = 34$ and $n = 64$)

| | Allergy | No allergy |
|---|---|---|
| Previous history of otitis externa | 62 % (21) | 56 % (36) |
| No history of previous otitis externa | 38 % (13) | 44 % (28) |

## DISCUSSION

The disease is common and often long lasting Therefore, correct treatment is important It has been demonstrated, for instance, that treatment by a combined agent, such as flumethasone pivalate + iodochlorohydroxyquinoline, is no better than placebo (Brahe Pedersen & Osterhammel 1971), and it is of the utmost importance not to use a drug which maintains or aggravates the disease, because of secondary development of allergy This possibility must be borne in mind in the event of lacking therapeutic effect or a localization outside the auditory meatus The use of neomycin preparations is particularly fraught with risk, but the possibility is present with all drugs Even steroids may sensitize due to their content of preserving agents, but Coskey (1965) reported two cases of allergy to hydrocortisone

The real incidence of allergic contact dermatitis among patients with otitis externa is prob-

Table VII *Bacteriological findings in swab material from the auditory canal in the two groups*

In some patients there was more than one bacteriological finding

| Bacterium | Allergy | No allergy |
|---|---|---|
| *Staph albus* | 11 (32 %) | 22 (34 %) |
| *Staph aureus* | 16 (47 %) | 24 (38 %) |
| *Str faec* | 4 (12 %) | 16 (25 %) |
| *Pseudomonas aeruginosa* | 9 (26 %) | 19 (30 %) |
| *E coli* | 1 (3 %) | 8 (13 %) |
| *Proteus* | — | 5 (8 %) |
| *Pfeiffer* | — | 1 (2 %) |
| *Miscellaneous* | 3 (9 %) | 7 (11 %) |

ably not 35%. Comparison of the history with the allergies demonstrated gave the following findings

1 In 12 of the 34 patients there was conformity between the allergy and the history The patients had observed flare-up or aggravation in connection with exposure

2 Ten patients had not observed any connection However, the agent concerned had been used and had failed to improve the condition

3 In the case of the last 12 patients there were no data in the history or else the allergy demonstrated could hardly be a contributory factor

According to the present study, allergic contact dermatitis occurs in 12–22% of patients suffering from otitis externa (group 1 + group 2)

## ZUSAMMENFASSUNG

Bei der Untersuchung von 98 an Otitis externa leidenden Patienten ergab eine Auswertung positive Läppchenproben bei 34 (35%) Die haufigsten Befunde waren medikamentelle Allergie, unter diesen Neomycin Auf grund der Untersuchung wird geschlossen, dass die Haufigkeit des allergischen Kontaktekzems bei an Otitis externa leidenden Kranken zwischen 12 und 22% liegt

## REFERENCES

Baumann, E S & Carr, C D 1961 Studies of factor considered responsible for diseases of the external auditory canal *Ann Otol 70*, 1055

Brahe Pedersen, C & Osterhammel, D 1971 Otitis externa treated with Locacorten Vioform ear drops *Ugeskr Laeg 133*, 389

Coskev, R J 1965 Contact dermatitis due to topical hydrocortisone and prednisolone *Michigan Medicine 64*, 669

McKelvie, M & McKelvie, P 1966 Some aetiological factors in otitis externa *Brit J Derm 78*, 227

Jones E H 1971 Allergy of the external ear *Otolaryng Clin North Amer 4*, 535

Singer, D E & Freeman E 1952 Otitis externa bacteriological and mycological studies *Ann Otol 61*, 317

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playing the sequence of intervals closely along the horizontal axis (for example, see Fig 2 a) This display was found to present a sensitive visual representation of the variations of inter-saccadic interval pattern As seen in this figure, peaks in the interval histogram tend to be associated with horizontal layers in the sequential display In cases in which the interval statistics show strong trends, as in caloric nystag mus (Figs 6 and 7) the sequential display often makes the trend readily visible and suggests how best to segment the data for histogram analysis (Fig 8)

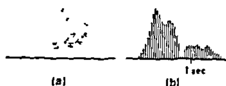



*Fig 4* Data from alcoholic nystagmus (*a*) Sequential display of interval duration 135 samples (*b*) Interval histogram 135 samples

## RESULT

The pattern of inter saccadic intervals of a spontaneous nystagmus recording is shown in Fig 2 The record was obtained from a patient suspected of having a labyrinthine lesion, nystagmus was found in all nine head positions in a routine clinical test In Fig 2 *a*, two characteristics of the inter-saccadic intervals can be observed (1) a weak trend in the interval durations is seen by the more frequent occurrence of the short intervals at the beginning of the record, whereas more intervals of longer duration occur in the second half of the record, and (2) the dots have a tendency to cluster into two layers, especially in the left half of the record This is reflected in the multi modal interval histogram obtained from the left half of the data and shown in Fig 2*b* The values of the second and third modes of the histogram are approximately twice and three times the value of the first mode

The results of analysis of positional nystag mus from another patient, suffering from post traumatic vertigo, are shown in Fig 3 Nystag mus was recorded in the caloric test position with the head tilted 30° to the right The trend in this record is quite pronounced especially at the beginning of the record It reflects the fact that the nystagmus was quite rapid when the head was first turned to the right, but gradually settled to a lower frequency The nystagmus recording shows a corresponding decline in intensity A gross layering effect can be seen in the sequential display, and histograms of the segmented data all show multi modal distributions The values of the higher order modes are approximately integral multiples of the value of the basic mode in each histogram even though the basic mode clearly increases with time

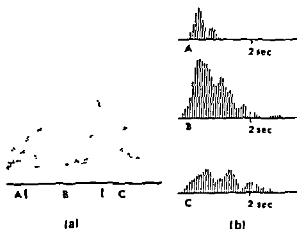



*Fig 3* Data from positional nystagmus (*a*) Sequential display of interval duration 370 samples (*b*) Interval histograms (A) 45 samples (B) 210 samples, (C) 115 samples

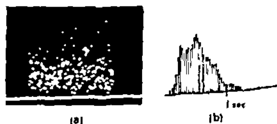



*Fig 5* Data from spontaneous nystagmus (*a*) Sequential display of interval duration 250 samples (*b*) Interval histogram 250 samples

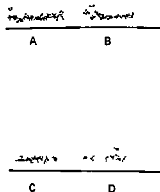



*Fig 6* Data from caloric nystagmus of a patient Sequential display of interval duration (A) Left cold (B) right cold (C) left hot (D) right hot Cold 30 C hot = 44 C water irrigation 30 seconds nystagmus recorded for 90 seconds after irrigation

The results in Fig 4 were obtained from a record of alcoholic positional nystagmus The subject, who weighed 150 pounds was found to be free from spontaneous positional or gaze nystagmus before the experiment Six ounces of 80 proof whisky were drunk by the subject within three minutes Thirty minutes later head turning to the left and to the right resulted in left and right beating nystagmus respectively which were recorded and analyzed Nystagmus was not so intense as that found in the first two cases and was often interrupted by erratic eye movements

So far the records have been selected to

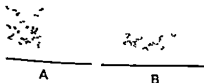



*Fig 7* Data from caloric nystagmus of a normal subject Sequential display of interval duration (A) Left cold (B) right cold Nystagmus recorded for 180 seconds after irrigation

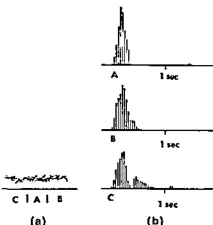



*Fig 8* Segmentation of data from caloric nystagmus (*a*) Sequential display of interval duration taken from Fig 6A (*b*) Interval histograms of segmented data (A) 60 samples (B) 70 samples (C) 75 samples

demonstrate the existence of multi modality However, in all three types of nystagmus namely spontaneous positional and alcoholic, there are records in which multi modality is not observed and the interval histograms are mono modal with varying dispersion For example Fig 5 shows the results from a spontaneous nystagmus record

In caloric nystagmus due to the rapid change in the intensity of nystagmus multi modality can generally be observed only at the beginning of the response Fig 6 shows sequential displays obtained from responses to four routine caloric irrigations in a patient suspected of having a central lesion The layering effect at the beginning of the first three records can clearly be observed The height of the top layer is approximately twice that of the bottom layer Fig 7 shows the results from two cold water irrigations in a normal subject The subject was free from spontaneous positional or gaze nystagmus Again layering effects at the beginning of the records are observed

In order to show that the characteristic change between interval histogram and nystagmus intensity (Cheng 1972 Cheng & Outerbridge 1973 *b*) also exist in vestibular nystagmus the data from the left cold irrigation of Fig 6 was segmented and the corresponding

interval histograms are shown in Fig 8 Fig 8 A corresponds to the middle portion of the nystagmus record where the nystagmus intensity was the highest The interval histogram is mono modal and symmetric Fig 8 B corresponds to the end portion of the nystagmus record where the intensity of nystagmus had declined from the peak value The interval histograms is mono modal but skewing towards the longer duration Fig 8 C corresponds to the beginning portion of nystagmus where the intensity was the weakest The interval histogram shows clearly the multi modal phenomenon

## DISCUSSION

Analysis of the inter saccadic intervals of caloric nystagmus indicates that the characteristic pattern of change of the interval distribution with intensity found in optokinetic nystagmus (Cheng, 1972, Cheng & Outerbridge, 1973 *b*) also occurs in vestibular nystagmus As nystagmus intensity decreases, the interval histogram changes from symmetric mono-modal, to asymmetric mono modal and finally to multi modal

In spontaneous, positional and alcoholic nystagmus, the inter saccadic interval histogram is mainly mono-modal with skewness towards the longer interval side or multi modal, whereas symmetric mono modal histograms have rarely been observed This may be due to the fact that in the records so far examined, nystagmus intensity has rarely reached that observed in caloric nystagmus It was also found that in some records (including caloric nystagmus), no multi-modal phenomenon was apparent in the entire record Again nystagmus intensity may be the reason It has also been shown that certain types of mental activity can have a randomizing effect on the saccadic rhythm (Cheng 1972)

The systematic variation of interval histogram with nystagmus intensity, clearly demonstrated in the case of optokinetic nystagmus (Cheng 1972, Cheng & Outerbridge, 1973 *b*) is also present in vestibular nystagmus together with the occurrence in both cases of multi modal histograms in which the modes are equally spaced, are of great interest Not only do these findings further emphasize that nystagmus generation mechanisms in the vestibulo ocular and optokinetic systems are very closely related, but a specific proposal can be made as to the type of neural mechanism which may be involved

Multi modal interval histograms similar to those described here have been observed in several neural systems (Poggio & Viernstein 1964, Herz et al, 1964, Bishop et al, 1964). Bishop et al suggested that multi modality could occur if a quasi periodic excitatory action potential process interacted synaptically with a random inhibitory process which intermittently blocked transmission of an excitatory action potential It will be noted that in such an interaction the output interval histogram would become mono modal if the mean frequency of the inhibitory process were reduced. It is quite plausible to postulate a similar interaction in the nystagmus generation mechanism, although the single inhibitory and excitatory neurons in the model of Bishop et al. would have to be replaced by groups of neurons, and the output action potential by a burst corresponding to each saccade Such a model has been found to be capable of simulating observed variations in the interval histogram of vestibular and optokinetic nystagmus (Cheng, 1972, Cheng & Outerbridge, 1973 *a*)

## ZUSAMMENFASSUNG

Mittels Digitalrechner wurden die Zeitintervalle zwischen dem Beginn konsekutiv schneller Komponenten beim vestibulären Nystagmus untersucht Die Kurvenverläufe der Histogramme der Zeitintervalle änderten sich mit der Intensität des Nystagmus analog zu den am optokinetischen Nystagmus berichteten Beobachtungen Während die Intensität des Nystagmus abnahm veränderten sich die Zeitverlaufskurven von anfänglich symmetrisch mono-modalen zu asymmetrisch mono-modalen und schließlich zu multi modalen Formen in denen die Moden höherer Ordnung ungefähr integrale Vielfache des ursprünglichen Modus

waren Diese Ergebnisse führen zu neuen Hypothesen mit Bezug auf die Nervengeflechte, die den vestibularen und optokinetischen Nystagmus hervorrufen

## REFERENCES


Bishop, P O, Levick, W R & Williams, W. O 1964 Statistical analysis of the dark discharge of lateral geniculate neurons *J Physiol* (Lond) *170*, 598

Cheng M 1972 Point process analysis in the physiological study of human nystagmus Ph D Thesis McGill University

Cheng M, Gannon, R P & Outerbridge, J S 1973 Frequency content of nystagmus *Aerospace Med 44* 383

Cheng M & Outerbridge, J S 1973 *a* A stochastic model for the inter saccadic interval sequence in ocular nystagmus Digest of 10th *Int Conf Med & Biol Engng* Dresden

— 1973 *b* Inter saccadic interval analysis of optokinetic nystagmus, *Vision Res*, in press

Herz, A, Greutzfeldt, O & Fuster, J 1964 Statistische Eigenschaften der Neuronenaktivitat im ascendierenden visuellen System *Kybernetik 2*, 61

Poggio, G F & Viernstein, L J 1964 Time series analysis of impulse sequences of thalamic sensory neurons *J Neurophysiol 27*, 517

Wilcock, M E & Kirsner, R L G 1969 A digital filter for biological data *Med Biol Engng 7*, 653



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interval histograms are shown in Fig 8 Fig 8 A corresponds to the middle portion of the nystagmus record where the nystagmus intensity was the highest The interval histogram is mono-modal and symmetric Fig 8 B corresponds to the end portion of the nystagmus record where the intensity of nystagmus had declined from the peak value The interval histograms is mono modal but skewing towards the longer duration Fig 8 C corresponds to the beginning portion of nystagmus where the intensity was the weakest The interval histogram shows clearly the multi modal phenomenon

## DISCUSSION

Analysis of the inter-saccadic intervals of caloric nystagmus indicates that the characteristic pattern of change of the interval distribution with intensity found in optokinetic nystagmus (Cheng, 1972, Cheng & Outerbridge, 1973 *b*) also occurs in vestibular nystagmus As nystagmus intensity decreases, the interval histogram changes from symmetric mono modal, to asymmetric mono modal and finally to multi modal

In spontaneous, positional and alcoholic nystagmus, the inter saccadic interval histogram is mainly mono-modal with skewness towards the longer interval side or multi modal, whereas symmetric mono modal histograms have rarely been observed This may be due to the fact that in the records so far examined, nystagmus intensity has rarely reached that observed in caloric nystagmus It was also found that in some records (including caloric nystagmus), no multi modal phenomenon was apparent in the entire record Again nystagmus intensity may be the reason It has also been shown that certain types of mental activity can have a randomizing effect on the saccadic rhythm (Cheng 1972)

The systematic variation of interval histogram with nystagmus intensity, clearly demonstrated in the case of optokinetic nystagmus (Cheng, 1972, Cheng & Outerbridge, 1973 *b*) is also present in vestibular nystagmus, t gether with the occurrence in both cases ( multi modal histograms in which the mod are equally spaced, are of great interest N only do these findings further emphasize that nystagmus generation mechanisms in the vestibulo ocular and optokinetic systems are very closely related, but a specific proposal can be made as to the type of neural mechanism which may be involved

Multi-modal interval histograms similar to those described here have been observed in several neural systems (Poggio & Viernstein 1964, Herz et al, 1964, Bishop et al, 1964) Bishop et al suggested that multi modality could occur if a quasi periodic excitatory action potential process interacted synaptically with a random inhibitory process which intermittently blocked transmission of an excitatory action potential It will be noted that in such an interaction the output interval histogram would become mono modal if the mean frequency of the inhibitory process were reduced. It is quite plausible to postulate a similar interaction in the nystagmus generation mechanism, although the single inhibitory and excitatory neurons in the model of Bishop et al would have to be replaced by groups of neurons, and the output action potential by a burst corresponding to each saccade Such a model has been found to be capable of simulating observed variations in the interval histogram of vestibular and optokinetic nystagmus (Cheng, 1972, Cheng & Outerbridge, 1973 *a*)

## *ZUSAMMENFASSUNG*

Mittels Digitalrechner wurden die Zeitintervalle zwischen dem Beginn konsekutiv schneller Komponenten beim vestibulären Nystagmus untersucht *Die Kurven*verlaufe der Histogramme der Zeitintervalle änderten sich mit der Intensitat des Nystagmus analog zu den am optokinetischen Nystagmus berichteten Beobachtungen Wahrend die Intensität des Nystagmus abnahm veranderten sich die Zeitverlaufskurven von anfanglich symmetrisch mono-modalen zu asymmetrisch mono-modalen und schließlich zu multi modalen Formen in denen die Moden hoherer *Ordnung* ungefahr integrale Vielfache des ursprunglichen Mode

a cat As the degree of nasal patency, however, is generally a measure of blood flow in the nasal mucosa (Malm, 1973), changes in patency were used to estimate the sensitivity of the nasal vessels to chemical agents The responses to intravenously administered drugs in a normally innervated or acutely denervated nose cavity were compared with those in a chronically denervated nose cavity in the same cat The nasal mucosae in 12 cats were sympathetically denervated by extirpation of one superior cervical ganglion A parasympathetic denervation was attained in 7 cats by extirpation of one pterygopalatine ganglion by a transpalatal approach In the latter case, however, sympathetic nerves are also cut Because of this not only the pterygopalatine ganglion on one side but also the superior cervical ganglia on both sides were extirpated At the denervations anaesthesia was induced with ether and continued with a short lasting barbiturate, Evipan®-Natrium (Bayer), given intracardially (about 20 mg/kg) Two to eight weeks later the cats were again anaesthetized, now with chloralose i v (about 80 mg/kg) after induction with ether The same anaesthesia was given to 6 normal cats The cats were tracheotomized and the body temperature was kept constant at 38°C by thermostat A water filled balloon, consisting of a very thin walled latex tube and tied at both ends over a polyethylene tube, was placed in each of the nose cavities (see Malm, 1973) Within the balloon there were small holes in the polyethylene tube, so that the pressure changes in the balloon could be transferred via the polyethylene tube to a transducer connected to an amplifier and a polygraph Each balloon was connected to a separate transducer and amplifier When the system was opened the pressure in the balloons was set to 6 cm of water and this resting level was controlled regularly

Lachrymal secretion was also estimated in the parasympathetically denervated cats using Schirmer's test Litmus papers were bent over the upper eyelids and a relative measure of the secretion was obtained from the degree of moistening of the papers

Phenylephrine, noradrenaline, adrenaline and methacholine were given in physiological saline solutions through a cannula in a femoral vein in doses of 0 0001, 0 001, 0 01, 0 1 and 1 μg/kg Noradrenaline was given to all the cats Phenylephrine and adrenaline were given to 5 of the cats which were only sympathectomized and to all the parasympathectomized cats Methacholine was given to the parasympathectomized cats in order to use Schirmer's test

## RESULT

The records showed both small rapid pressure changes with the same frequency as the heart beats and slower and bigger accompanying the respiration These and the effect of noradrenaline intravenously are shown in Fig 1 Phenylephrine, noradrenaline and adrenaline always decreased the amplitude of the small rapid pressure changes and increased the nasal patency In a few of the cats, where the blood pressure was recorded, the bigger doses of the drugs evoked a blood pressure rise and this might influence the responses of the drugs on the nose Blood pressure changes could have been avoided or reduced by giving the agents intra-arterially close to the nose cavities, but as the dosages of the agents then run the risk of being unequal in the two cavities, the intravenous way of administration was chosen

*Comparisons between two normally innervated nose cavities*

The changes in nasal patency to chemical agents in two normally innervated nose cavities in the same cat will not necessarily be equal Anatomical differences may exist and the positions and sizes of the two balloons may vary Efforts were made to reduce these latter sources of error The positions were adjusted with the aid of markings on the polyethylene tubes Two balloons were always chosen with as equal volumes of water as possible at the same pressure Nevertheless, it was desirable to compare the effects in two normally innervated nose cavities after vasoactive agents The effects of a series of doses of noradrenaline intravenously were

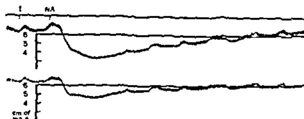



*Fig 1* Pressure variations in two water filled balloons, one (*upper record*) located in a chronically sympathectomized and one (*lower record*) in a normally innervated nose cavity of a cat Downward deflexion indicates a decreased pressure in a balloon caused by an increased patency Calibration pressure in cm of water Time mark in seconds Noradrenaline 1 μg/kg i v is given at NA The start of an inspiration is marked with an I

The mean differences with S E M between the two sides in 6 cats were for 0 01 μg/kg 0 01 ± 0 03 cm of water, for 0 1 μg/kg 0 02 ± 0 07 and for 1 μg/kg 0 11 ± 0 19

*Sensitivity after sympathetic denervation*

Twelve cats were investigated 2 to 8 weeks after extirpation of the superior cervical ganglion on one side At first the responses to the drugs in both cavities were recorded with the control side innervated Then the cervical sympathetic nerve of that side was divided and the responses again recorded Finally the superior cervical ganglion was extirpated and the effects of the drugs on the acutely and chronically sympathectomized sides compared

Nine of the cats were given noradrenaline on all three test occasions and the mean values of the pressure changes after different doses are shown in Fig 2 When the control side was normally innervated the mean differences at paired comparisons between the two sides were significant for 1 ($p < 0\ 001$), for 0 1 ($p < 0\ 01$) and for 0 01 μg/kg ($p < 0\ 02$) but not for 0 001 μg/kg ($p > 0\ 05$) The threshold doses between the two sides also differed ($p < 0\ 02$)

After the cervical sympathetic nerve on the control side was acutely cut, the mean responses on the two sides approached one another more closely, but still the mean pressure changes were bigger on the chronically ganglionectomized side The mean differences were however not significant for any of the doses After acute extirpation of the superior cervical ganglion the mean responses were about the same as after cutting the cervical sympathetic nerve The mean values of the acutely ganglionectomized sides of the 9 cats, which also are seen in Fig 2, were not significantly different from the values of the chronically denervated ones Three cats were given noradrenaline only when both sides were sympathectomized, one acutely and one chronically The differences were not significant when the values from these 3 cats were included

A supersensitivity may be shown by changes in threshold doses The mean doses of noradrenaline giving just noticeable responses were smaller in the chronically than in the acutely sympathectomized sides, but the differences at paired comparisons were not significant The other drugs were given to 5 of the cats and their differences were not significant either, as seen in Table I

Another sign of supersensitivity may be an increase of the durations of the responses On the chronically sympathectomized side the pressure responses after noradrenaline seemed to return to the resting pressure later than on the intact (see Fig 1) or the acutely operated side The mean time to return to resting pressure was significantly longer in the chronically than in the acutely sympathectomized sides (see Table II)

In 4 of the 12 cats the pressure was recorded when the acute preganglionic and postganglionic sympathetic denervations were done It was easily observed on the record that within a quarter of a minute after the decentralization the pressure increased in the balloon on the acutely operated side and remained on a higher level No further increase could be seen when the superior cervical ganglion had been extirpated The pressure on the previously operated side did not change

*Sensitivity after parasympathetic denervation*

The sensitivity of the nasal mucosa was investigated in 7 cats 3 to 7 weeks after extirpation of the pterygopalatine ganglion on one side and the superior cervical ganglion on both sides The

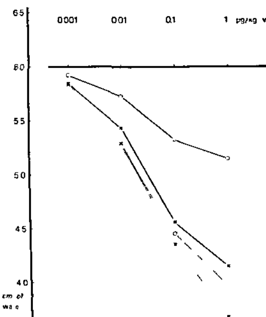



*Fig 2* The mean responses to noradrenaline in normally innervated (O—O) and in chronically sympathectomized nose cavities (O- O) in 9 cats, and in the previously intact cavities after acute sympathectomy (× — ×) tested at the same time as the chronically sympathectomized (×—×) *Abscissa* doses of noradrenaline i v *Ordinates* pressure in cm of water The resting pressure was 6 cm of water

same drugs and doses were given twice and the two sets of balloons, transducers and amplifiers changed place between the tests This was done in order to reduce the effects of variations in the balloon sizes and positions The mean of the two tests was taken as a response to a drug The mean values of the 7 cats were larger for the parasympathetically denervated sides, but paired

Table I *The mean threshold doses ± S E M in µg/kg in acutely and chronically sympathectomized nose cavities*

| | Phenyl ephrine | Nor adrenaline | Adrenaline |
|---|---|---|---|
| Acutely op side | 0 26±0 18 | 0 022±0 011 | 0 0010±0 0000 |
| Chronically op side | 0 26±0 19 | 0 021±0 011 | 0 0008±0 0002 |
| Differences at paired comparisons | $p > 0\ 05$ | $p > 0\ 05$ | $p > 0\ 05$ |
| No of cats | 5 | 12 | 5 |

Table II *The mean durations ± S E M in seconds of the responses to noradrenaline in acutely and chronically sympathectomized nose cavities*

| Dose in µg/kg | 0 01 | 0 1 | 1 |
|---|---|---|---|
| Acutely op side | 36±7 | 70±9 | 73±12 |
| Chronically op side | 45±8 | 85±9 | 126±11 |
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comparisons showed that the mean differences between the sides were not significant The threshold doses and the durations of the responses to higher doses between the sides were not significantly different

The secretory nerve fibres to the lachrymal gland in the cat originate in or pass through the pterygopalatine ganglion (Botelho et al, 1966) To examine if the lachrymal gland on the transpalatally operated side had developed a supersensitivity, Schirmer's test was used As evidence of an extirpation of the pterygopalatine ganglion, the litmus papers became more moistened on the parasympathetically denervated side following supraliminal doses of methacholine in all the cats

## DISCUSSION

Malcomson wrote in 1959 "In the sympathectomized nose, the reaction to minute traces of adrenaline intravenously appears earlier than on the intact side " This statement was the starting point for the present study

To investigate the effects of chronic denervation on the nasal vessels a method using intranasal balloons was chosen as it was simple, gave regular records for many hours and seemed to be as sensitive as the rhinomanometric method used by Malcomson (1959) A disadvantage was that the pressure of the balloons might stimulate afferent nerve fibres Very little is known, however, of the properties of such nerve fibres in the nasal mucosa It is possible that there are epithelial receptors which adapt rapidly, just as do receptors in the epipharynx (Nail et al, 1969) Another disadvantage of the method was that

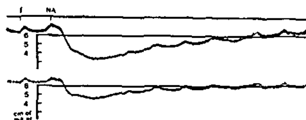



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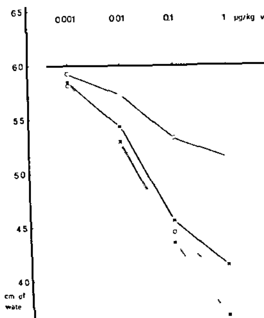



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the two balloons in each cavity in a cat could not be exactly equal in size and position The responses of noradrenaline from two normally innervated nose cavities in the same cat showed, however, that comparisons may be done

Acute sympathetic denervation decreased the nasal patency probably due to a dilatation of the blood vessels in the mucosa If the decreased resting tone of the vessels remains for weeks after the denervation this state may be the cause of the bigger responses evoked by noradrenaline on the denervated side compared to the normally innervated A dose given to dilated vessels may decrease the blood flow more than if the same dose is given to vessels with normal tone The amount of a drug per unit of time arriving at the smooth muscles of the vessels may increase with increasing blood flow The responses may also be bigger due to supersensitivity The part played by the latter cause cannot, however, be determined with the method used

When the results from a chronically and an acutely denervated side in the same cat are compared, the answer is easier to give If the tone of the vessels did not change at all during the 2 to 8 weeks after the initial change following extirpation of the sympathetic ganglion the differences of the responses between the acutely and the hronically denervated side must be due solely to supersensitivity If the tone changed, however, it was certainly in the direction of a regain of tone i e the vessels in the chronically sympathectomized side were less dilated than in the acutely and consequently bigger and longer responses from the former must still be a manifestation of supersensitivity The differences in the durations of the responses to noradrenaline when a chronically and an acutely sympathectomized side were compared thus seem to be caused by supersensitivity in the chronic side As the responses following noradrenaline were not significantly bigger in a chronically than in an acutely sympathectomized side a supersensitivity is probably only of minor importance to the differences in responses to the same drug in a chronically denervated compared to a normal side

The drugs affected the vessels, but they may also evoke secretion If this secretion is not carried away the nasal patency decreases The balloons and polyethylene tubes, however, did not obstruct the natural removal of the secretion towards the epipharynx Furthermore if the nasal glands have about the same sensitivity as the salivary glands (Emmelin & Muren 1951) the doses of the drugs probably evoke no or very little secretion Recently evidence has been given that stimulation of the vidian nerve evokes a secretion which can be abolished by atropine intravenously and by nicotine applied to the pterygopalatine ganglion (Eccles & Wilson 1973) Thus supersensitivity may develop after extirpation of that ganglion If the change of sensitivity of the nasal glands to the vasoconstrictor drugs after denervation is about the same as of salivary glands (Emmelin & Muren 1951) it is still unlikely that at least lower doses of the drugs evoke secretion

The results thus imply that the bigger responses to noradrenaline on the chronically sympathetically denervated nasal vessels compared with the normal vessels are probably only to a minor degree caused by supersensitivity On the other hand a supersensitivity seems to be the cause of the longer responses on a chronically compared with an acutely sympathectomized mucosa It may be a sign of only a small change in sensitivity of the nasal vessels that the threshold doses and the magnitude of the responses to supraliminal dosages did not differ between the chronically and the acutely sympathectomized sides No supersensitivity could be demonstrated in any respect between sides that were both parasympathetically and sympathetically denervated when compared with those that were only sympathetically denervated

A study of the effects of a preganglionic parasympathetic denervation, which is a part of the denervation done in patients with vasomotor rhinitis was not performed here The change in sensitivity after such a denervation however ought to be still less than after a postganglionic in accordance with Cannon's law of denervation

## ZUSAMMENFASSUNG

In sympathisch und parasympathisch denervierten Nasenschleimhauten von 19 Katzen wurde der Effekt von Phenylephrin, Noradrenalin und Adrenalin auf die Blutgefasse mit Hilfe von wassergefullten Ballons, eingefuhrt in die Nasenraume, registriert Einer der Nasenraume diente als Kontrolle in der gleichen Katze Der Effekt von Noradrenalin war bedeutend grosser auf der Seite, wo 2–8 Wochen zuvor das Ganglion Cervicale Superior entfernt worden war Diese Unterschiede beruhen aber nicht nur auf Übersensibilitat, sondern sind wahrscheinlich auch ein Effekt erweiterter Blutgefasse in der denervierten Nasenschleimhaut Wenn beide Seiten denerviert wurden, die eine chronisch, die andere akut, waren die Antworten nur dann verschieden, wenn die Dauer verglichen wurde, diese Unterschiede scheinen auf Übersensibilitat zu beruhen Keine bemerkenswerte Unterschiede, weder mit Schwellendosen noch mit Überschwellendosen der Substanzen, konnten gezeigt werden, wenn die postganglionaren parasympathischen Nerven durchschnitten worden waren

## REFERENCES

Botelho, S Y , Hisada, M & Fuenmayor, N 1966 Functional innervation of the lacrimal gland in the cat *Arch Ophthal* (Chic ) *76*, 581

Cannon, W B & Rosenblueth, A 1949 *The supersensitivity of denervated structures* Macmillan, New York

Chandra, R 1969 Transpalatal approach for Vidian neurectomy *Arch Otolaryng* (Chic ) *89*, 126

Chasin, W D & Lofgren, R H 1967 Vidian nerve section for vasomotor rhinitis *Arch Otolaryng* (Chic ) *86*, 103

Dahlstrom, A & Fuxe, K 1965 The adrenergic innervation of the nasal mucosa *Acta Otolaryng* (Stockh ) *59*, 65

Duff, R S 1952 Effect of sympathectomy on the response to adrenaline of the blood vessels of the skin in man *J Physiol* (Lond ) *117*, 415

Eccles R & Wilson, H 1973 The parasympathetic secretory nerves of the nose of the cat *J Physiol* (Lond ) *230* 213

Emmelin, N & Muren, A 1951 Sensitization of the submaxillary gland to chemical stimuli *Acta Physiol Scand* (Stockh ) *24*, 103

Golding-Wood, P H 1961 Observations of petrosal and vidian neurectomy in chronic vasomotor rhinitis *J Laryng 75*, 232

— 1962 Pathology and surgery of chronic vasomotor rhinitis *J Laryng 76*, 969

Hiranandani, N L Jr 1966 Treatment of chronic vasomotor rhinitis with clinico-pathological study of Vidian nerve section in 150 cases *J Laryng 80*, 902

Ishii, T 1970 The cholinergic innervation of the human nasal mucosa *Pract Otorhinolaryng* (Basel) *32*, 153

Lindgren, P 1955 The mesencephalon and the vasomotor system *Acta Physiol Scand* (Stockh ), Suppl *121*, 1

Malcomson, K G 1959 The vasomotor activities of the nasal mucous membrane *J Laryng 73*, 73

Malm, L 1973 Stimulation of sympathetic nerve fibres to the nose in cats *Acta Otolaryng* (Stockh ) *75*, 519

Minnis, N L & Morrison, A W 1971 Transseptal approach for Vidian neurectomy *J Laryng 85*, 255

Nail, B S , Sterling, G M & Widdicombe, J G 1969 Epipharyngeal receptors responding to mechanical stimulation *J Physiol* (Lond ) *204*, 91

Nomura, Y & Matsuura, T 1972 Distribution and clinical significance of the autonomic nervous system in the human nasal mucosa *Acta Otolaryng* (Stockh ) *73*, 493

Ohlin, P & Stromblad, B C R 1958 Supersensitivity of the vessels of the parotid gland after denervation *Brit J Pharmacol 13*, 227

Tschalussow, M A 1913 Die Innervation der Gefässe der Nasenschleimhaut *Pflueger Arch Ges Physiol 151*, 523

Ward, Jr, G R , Pearson, R N & Ederstrom, H E 1967 Catecholamine sensitivity of cat leg vessels after sympathectomy *Amer J Physiol 212*, 466

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# BLOOD GROUP SUBSTANCE A IN CARCINOMAS OF THE LARYNX

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*Abstract* Tissue from 9 squamous cell carcinomas of the larynx were investigated for the presence of blood group antigen A The antigen reactivity in the carcinomas was compared, by titration, with the reactivity of adjacent normal epithelium in the same specimen Normal epithelium reacted positively in all cases except one In this case the normal epithelium contained areas showing no reactivity for blood group antigen In 4 out of 9 cases the malignant cells gave completely negative reaction In 4 specimens there was a patchy distribution of positive reacting cells within the carcinoma In one specimen the majority of cells in the carcinoma showed no decrease in reactivity

The demonstration of blood group substances A and B in cells and tissues other than the erythrocytes has a long history (Ladsteiner & Levine, 1926, Kritschewski & Schwartzmann, 1927) In the squamous epithelial cells of the normal vocal cords, their localization on the cell membranes has been demonstrated by use of an immunofluorescence staining technique and the mixed cell agglutination reaction (Szulman, 1960, Kovarik 1968)

It is well established that many different types of human tumours have tumour associated antigens (Gold & Freedman 1965, Hellström et al, 1971) Furthermore, several studies have indicated that acquisition of a tumour antigen is accompanied by loss of normal cytoplasmic membrane antigens (Burtin et al, 1972)

Partial or complete loss of blood group antigens is reported to occur in premalignant and malignant diseases developing from tissues in which they are normally present, these include tissue of the bladder (Kay, 1957), oral mucosa (Dabelsteen & Pindborg, 1973), cervix, stomach (Davidsohn et al, 1966), and pancreas (Davidsohn et al 1971), and it has been suggested by some of these authors that the disappearance of antigen is an early sign of malignant transformation

Kovarik (1968) showed that six of nine carcinomas from the larynx reacted negatively for isoantigen A or B, the rest reacted positively as normal epithelium A recent publication (Dabelsteen, 1972) states that the amount of blood group antigen A in normal oral mucosa varies from person to person and that sometimes the non-secretor group (Hartmann, 1941) has so little antigen that it can hardly be measured Any estimation of the reactivity of blood group antigen in carcinomas should therefore be compared with the reactivity in histologically normal mucosa This was not done in previously published work

The present paper describes the distribution of blood group antigen in carcinomas of the larynx and compares the reactivity of the blood group antigens in the carcinoma with the reactivity in histologically normal epithelium adjacent to the carcinoma

## MATERIAL AND METHODS

The material consists of surgical specimens from 9 male patients laryngectomized on account of

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Table I *Controls for establishing specificity of immunoperoxidase and immunofluorescence staining*

| Blood group substance | Blood group antisera | Conjugate | Results |
|---|---|---|---|
| A | Phosphate-buffered saline | Labelled antiglobulin | Negative |
| A | Anti B | Labelled antiglobulin | Negative |
| A | Anti A absorbed with $A_1$ erythrocytes | Labelled antiglobulin | Negative |
| B | Anti A with known reactivity | Labelled antiglobulin | Negative |
| A[a] | Anti A with known reactivity | Unlabelled antiglobulin followed by peroxidase reaction | Negative |
| A | Anti A with known reactivity | Labelled antiglobulin | Positive |

[a] For immunoperoxidase stainings only

squamous cell carcinomas of the larynx All patients belonged to blood group A In all specimens histologically normal mucosa was found adjacent to the tumours Before surgery all carcinomas had been treated by irradiation

Biopsies of five of the carcinomas taken before irradiation were also investigated Histologically normal mucosa was present in four of these biopsies

The surgical specimens as well as the biopsies were fixed in 10% formalin, embedded in paraffin and sectioned at 5 μm Blood group substances were detected in the epithelium by two different immunological staining procedures, the immunoperoxidase (IP) method (Avrameas, 1970) and the immunofluorescence (IF) method (Weller & Coons, 1954, Dabelsteen & Fulling 1971)

Both staining methods were used as double-layer techniques, the first layer being a human blood group test serum and the second layer a rabbit antihuman IgG globulin conjugated with peroxidase for the IP staining and with fluorescein isothiocyanate (FITC) for the IF staining The blood group antiserum was provided by Statens Seruminstitut, Copenhagen and the conjugates were purchased heavy chain specific from Dakopatts A-S Copenhagen

The peroxidase conjugate had an antihuman IgG titre of 300 (i e 1 ml conjugate absorbs 300 μg pure IgG) The molar peroxidase/protein ratio was 1 20 A working titre of 1 20 was found by chessboard titration

The FITC conjugate had an antihuman IgG titre of 200 The mean optical density ratio 495/280 nm was 0 66 with 0 30 as lowest and 0 95 as highest ratio (Unconjugated immunoglobulin molecules with an O D ratio below 0 3, and conjugated immunoglobulins with an O D ratio above 0 95, were removed by ion exchange chromatography ) By chessboard titration (Wick & Beutner, 1970) a working titre at 1 40 was found To ensure that the IF and IP stainings were specific, control reactions were made These are summarized in Table I

The fluorescence microscope used was a Leitz Orthoplan with a darkfield oil immersion condenser The primary filter was a FITC-interference filter with red contrast band (Rygaard & Olsen, 1969) The secondary filter was a 2 mm glass filter (Schott & Gen, Germany) matched to fit the primary filter (Rygaard & Olsen, 1971)

The amount of blood group antigen in the epithelium was estimated by a two-fold serial dilution titration, and the reciprocal of the highest dilution giving positive reaction was regarded as the endpoint titre The reactions were read as positive or negative

The surgical specimens were all titrated in the IP staining The biopsies and the corresponding

surgical specimens were stained simultaneously with the IF staining

## RESULTS

In all 5 cases where it was possible to compare irradiated tissue with tissue from the same patient before irradiation, no difference in reactivity for blood group antigen was found Furthermore, although the end-point titres are different in the IP and the IF staining reactions, the reaction patterns are identical (Table II)

### *Blood Group Antigen Reactivity in Laryngeal Carcinomas and Adjacent Normal Epithelium (IP Stainings)*

*Normal epithelium*

In all cases except one the normal mucosa reacted positively for blood group antigen A (Table III) The positive reaction was seen as a uniform brown staining of the cell membranes in the spinous cell layer, the basal cell layer and the superficial cell layers do not react (Fig 1)

In one case (no III) the normal epithelium contained areas showing no reactivity for blood group antigen (Fig 1)

*Squamous cell carcinoma*

Three different patterns of reaction were seen in carcinomas

(1) In 4 of the 9 cases the squamous cell carcinoma reacted negatively with all concentrations of anti-A serum (Table III, Fig 2)

(2) In 4 cases about one-third of the cells in the carcinoma reacted positively for blood group antigen The positive cells were arranged in small groups unevenly distributed within the carcinoma (Fig 3) The positive cells in three of these cases had endpoint titres of the same magnitude as the normal adjacent epithelium In one case (no III) the end-point titre of the cancer cells was lower than that of the adjacent normal epithelial cells

(3) In 1 case (no IV) all the tumour cells reacted positively with end-point titres which were higher than those obtained in normal adjacent epithelium (Fig 4)

Finally, in 2 cases where epithelium adjacent to the carcinoma exhibited slight dysplasia no change in blood group antigen was noted

Table II *End point titres obtained in immunofluorescence staining of non irradiated and irradiated laryngeal mucosa*

– negative
n t = not tested
The numbers in Tables II and III refer to the same patients

| Patient no | Biopsy non irradiated | | Surgical specimens irradiated | |
|---|---|---|---|---|
| | Normal | Tumour | Normal | Tumour |
| I | 1 32 | | 1 32 | |
| II | n t | | 1 4 | |
| III | 1 32 | 1 8 | 1 32 | 1 8 |
| IV | n t | 1 32 | Conc | 1 32 |
| V | 1 8 | | 1 8 | |

Table III *End-point titres obtained by staining normal epithelium and laryngeal carcinomas in an immunoperoxidase staining method*

– = negative
n t = not tested
The numbers in Tables II and III refer to the same patients

| Patient no | Normal epithelium | Tumour | |
|---|---|---|---|
| | | Most cells | Patchy areas |
| I | 1 16 | – | – |
| II | Conc | – | – |
| III | 1 8 | – | Conc |
| IV | Conc | 1 16 | – |
| V | 1 4 | – | – |
| VI | 1 8 | – | 1 8 |
| VII | Conc | – | Conc |
| VIII | 1 4 | – | 1 8 |
| IX | Conc | – | – |

## DISCUSSION

The present work evaluates the amount of blood group antigen A in 9 squamous cell carcinomas of the larynx by making comparison with the amount of antigen in normal mucosa of the same patient It shows that in 4 of the cases there was a total loss of blood group antigen

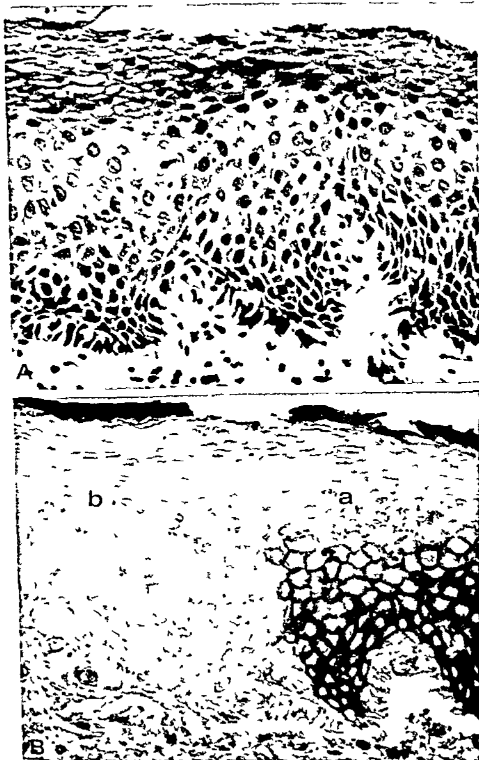



*Fig 1* Normal squamous epithelium adjacent to laryngeal carcinoma (A) Haematoxylina and eosin staining (B) Immunoperoxidase staining of neighbouring section Black intercellular spaces indicate positive reacting cells (*a*) normal distribution of blood group antigen (*b*) loss of antigen ×200

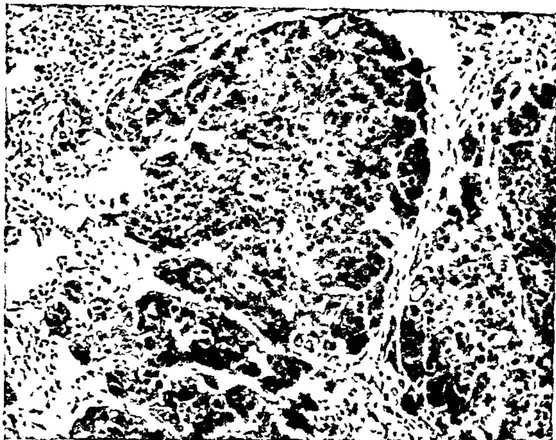


*Fig 2* Squamous cell carcinoma of the larynx. Immunoperoxidase staining followed by haematoxylin and eosin staining All cancer cells react negatively for blood group antigen ×77

the carcinomas In 4 other cases there was a decrease in reactivity of blood group antigen illustrated by the patchy distribution of positive cells In 1 case (no IV) there was no decrease of blood group antigen, on the contrary, this case demonstrated a stronger reaction in the carcinoma than in the adjacent mucosa

The findings in the present study are in agreement with the previous study of A and B antigen in carcinomas of the oral mucosa. However, the titration of anti-A serum, which has proven useful in previous work (Dabelsteen & Pindborg, 1973) is of value in only one of the present series In this case the normal epithelium adjacent to the tumour and the tumour cells both reacted positively with the higher concentrations of antibody but only the normal cells showed reactivity for blood group antigen A when the anti-A serum was diluted

It is of interest to notice that 2 cases exhibiting epithelial dysplasia adjacent to the cancer showed no decrease in blood group antigen A reactivity This is in contrast to previous studies which have shown a decrease in quantity of blood group antigen in lesions exhibiting epithelial dysplasia, including lesions of the oral mucosa (Dabelsteen & Fulling, 1971) and the mucosa of the uterine cervix (Davidsohn et al, 1969) As some, but not all, lesions exhibiting epithelial dysplasia develop into cancer, it would be of great interest to know how the change in blood group antigen reactivity is correlated to the prognosis of the lesions

The relatively large number of cases containing patchily distributed areas of cancer cells reacting positively for blood group antigen A indicates that the change in reactivity of A antigen is a tumour-associated change rather

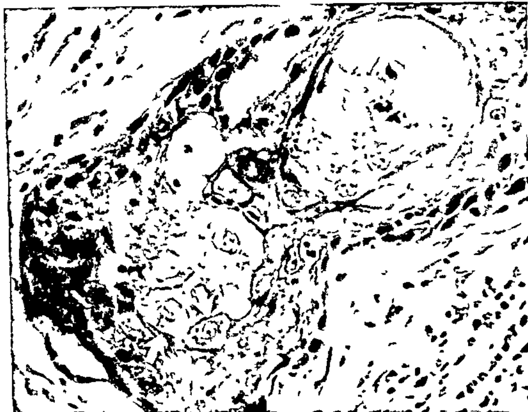


*Fig 3* Squamous cell carcinoma of the larynx Immunoperoxidase staining followed by haematoxylin and eosin staining Area showing patchy distribution of blood group antigen Black intercellular spaces (*arrow*) indicate presence of blood group antigen ×250

than tumour specific This of course decreases the diagnostic value of change in blood group antigen reactivity for laryngeal epithelium with suspected malignant properties No attempt has in the present study been made to correlate the presence or absence of staining with the biological behaviour of the tumour This seems a worthwhile investigation to continue with It has been mentioned (Davidsohn 1972) that no other disease is presently known in which the iso antigens A and B are lost to the same extent and with the same regularity as in carcinomas, this problem needs very careful investigation before the diagnostic value of decrease in epithelial blood group antigen can be properly evaluated

Several studies have demonstrated antigenic changes of the cell membrane during malignant transformation These changes include appearance of new antigens as well as loss of normal cytoplasmic membrane antigen Whether the loss of blood group antigen in carcinomas of the larynx is connected with appearance of new antigen is unknown Furthermore it would be of interest to know whether the positive cancer cells are really positive for blood group antigen or whether a cross reactivity takes place between blood group antigen A and a tumour antigen That this is possible has recently been demonstrated by Gold et al (1972)

In conclusion the present study has shown that the number of blood group antigen A positive cells is appreciably reduced in most laryngeal carcinomas Furthermore it has shown that this is a tumour associated change and not a tumour-specific change



*Fig 4* Squamous cell carcinoma of the larynx (A) Haematoxylin and eosin staining (B) Immunoperoxidase staining of neighbouring section Black intercellular spaces indicate positive reacting cells Note that nearly all cancer cells react positively · 77

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## ZUSAMMENFASSUNG

Gewebe von 9 Larynx Plattenepithelkarzinomen wurden auf das Vorkommen von Blutgruppe A Antigen hin untersucht Die Antigen Reaktionsfahigkeit in den Karzinomen wurde mittels Titrieren mit der Reaktionsfahigkeit angrenzenden Normalepithels bei demselben Praparat verglichen Das normale Epithel reagierte mit einer [illegible]

von positiv reagierenden Zellen im Karzinom In einem Fall zeigte ein Mehrzahl der Zellen im Karzinom keine Verminderung der Reaktionsfahigkeit

## REFERENCES

Avrameas, S 1970 Immunoenzyme techniques Enzymes as markers for the localization of antigens and antibodies *Int Rev Cytol 27*, 349

Burtin P Clausell, D, Bora, J & von Kleist S 1972 Variations of membrane antigens of human epithelial cells at different stages of differentiation and in malignancy In *Cell differentiation* (ed R Harris, P Allen & C Viza) Munksgaard, Copenhagen

Dabelsteen, E 1972 Quantitative determination of blood group substances A of oral epithelial cells by immunofluorescence and immunoperoxidase methods *Acta Path Microbiol Scand*, Sect A, *80*, 837

Dabelsteen, E & Fulling, H -J 1971 A preliminary study of blood group substances A and B in oral epithelium exhibiting atypia *Scand J Dent Res 79*, 387

Dabelsteen, E & Pindborg, J J 1973 Loss of epithelial blood group substances A in oral carcinomas *Acta Path Microbiol Scand*, Sect A, In press

Davidsohn I 1972 Early immunologic diagnosis and prognosis of carcinoma *Amer J Clin Path 57*, 715

Davidsohn I, Kovarik, S & Lee, C L 1966 A, B, and O substances in gastrointestinal carcinoma *Arch Path* (Chic) *81*, 381

Davidsohn I, Kovarik, S & Ni L Y 1969 Isoantigens A, B, and H in benign and malignant lesions of the cervix *Arch Path* (Chic) *87*, 306

Davidsohn, I, Ni, L Y & Stejskal, R 1971 Tissue isoantigens A, B and H in carcinoma of the pancreas. *Cancer Res 31*, 1244

Gold, J M, Freedman, S O & Gold, P 1972 Human anti CEA antibodies detected by radioimmunoelectrophoresis *Nature New Biol 239*, 60

Gold, P & Freedman, S O 1965 Demonstration of tumor specific antigens in human colonic carcinoma by immunological tolerance and absorption technique *J Exp Med 121*, 439

Hartmann, G 1941 *Group antigens in human organs* Munksgaard Copenhagen

Hellstrom, I, Hellstrom, K E, Sjogren, H O & Warner, G A 1971 Demonstration of cell mediated immunity to human neoplasms of various histological types *Int J Cancer 7*, 1

Kay, H E M 1957 A and B antigens of normal and malignant cells *Brit J Cancer 11*, 409

Kovarik S 1968 ABO antigens in cancer Detection with the mixed cell agglutination reaction *Arch Path* (Chic) *86*, 12

Kritschewski, L. & Schwartzmann L 1927 Die gruppen specifische Differentierung der menschlichen Organe *Klin Wschr 6*, 2081

Landsteiner, K & Levine, P 1926 On group specific substances in human spermatozoa *J Immun 12*, 415

Rygaard, J & Olsen, W 1969 Interference filters for improved immunofluorescence microscopy *Acta Path Microbiol Scand 76*, 146

— 1971 Determination of characteristics of interference filters *Ann N Y Acad Sci 177*, 430

Szulman, A E 1960 The histological distribution of blood group substances A and B in man *J Exp Med 111*, 785

Weller, T H & Coons, A H 1954 Fluorescent antibody studies with agents of varicella and herpes zoster propagated in vitro *Proc Soc Exp Biol Med 86*, 789

Wick, G & Beutner, E H 1970 Indirect immunofluorescence chessboard titrations of bullous pemphigoid sera Relationship between antibasement membranes antibody titres and antigenic substrate In *Standardization in immunofluorescence* (ed E J Holborow), p 185 Blackwell Scientific Publications Oxford and Edinburgh

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# THE EFFECTIVENESS OF A STERNOMASTOID MUSCLE FLAP IN PREVENTING POST-PAROTIDECTOMY OCCURRENCE OF THE FREY SYNDROME

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(Received June 28 1973)

*Abstract* Among over 350 parotidectomies the effectiveness of a pedicled sternomastoid muscle flap in preventing post parotidectomy occurrence of the Frey Syndrome was tested during the past year in a random sampling of 35 patients with such a muscle flap and 35 control patients Results of the study indicate that the muscle flap, although improving cosmetic defects associated with surgery is incapable of inhibiting the Frey Syndrome and that other means must yet be devised to prevent the occurrence of gustatory sweating following parotid surgery

The Frey, or auriculotemporal, Syndrome is an unusual clinical problem which involves facial flushing or sweating following parotid gland infection surgery or trauma Several theories have been put forward concerning the mechanism by which this syndrome occurs, but the most plausible explanation appears to be misdirection of parasympathetic secretomotor fibres from the auriculotemporal nerve (which under normal circumstances provides secretomotor fibres to the parotid gland) to sweat glands or small vessels overlying the site of the gland (Blumenfeld & Friedman, 1967, Smith et al, 1970) Subsequent stimulation of the nerve results in sweating or flushing of the face The complication following surgery of the parotid gland has been estimated to be 25–97% (Hunt et al, 1966) although most patients do not require medical attention

Since 1964, over 350 parotidectomies have been performed at the E N T Clinic of the University of Göttingen A pedicled flap from the sternomastoid muscle was utilized in selected patients in order to prevent occurrenc of the Frey Syndrome following parotid surgery It was thought that this muscle flap would ac as a "barrier" to inhibit the misdirection of th auriculotemporal fibres subsequent to surgery The present study was a critical analysis of th effectiveness of this sternomastoid muscle fla in preventing post-parotidectomy occurrence c the Frey Syndrome

## MATERIALS AND METHODS

As previously noted, a sternomastoid muscl flap was utilized to cover the operative defect in selected patients undergoing parotid surgery for non malignant disease This muscle flap was based superiorly, and originated from the insertion of the sternomastoid muscle on the mastoid process (Fig 1) A thick muscle pedicle could then be sutured over the main trunk and primary rami of the facial nerve to cover the site of emergence of the auriculotemporal nerve into the retromandibular fossa

A random sampling of 35 patients with a sternomastoid muscle flap as described and a control group of 35 patients without such a flap were examined for incidence and severity of the Frey Syndrome following surgery

Of the patients studied, parotid surgery had been performed between 1 and 6 years previously The essential postoperative course of these patients had been without significant consequence

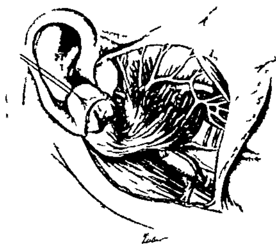


*Fig 1* Pedicled sternomastoid muscle flap used to cover operative defect in patients undergoing parotid surgery

A profile of all patients studied is given in Table I

All patients were questioned concerning subjective symptoms related to the Frey Syndrome These questions involved asking whether or not the patients noticed facial warmth, flushing or sweating in association with such gustatory stimuli as eating, smoking or use of cholinergic medications The Minor starch iodine test was then performed, as described by Laage-Hellman (1957) An alcohol iodine oil solution was prepared by mixing 3 grams of iodine with 20 grams of castor oil, and bringing the final solution to 200 grams by the addition of absolute alcohol Both lateral portions of each patient s face and upper regions of the neck were painted with this preparation, and the solution allowed to dry These areas so prepared were then lightly covered with starch powder Lemon slices were employed as stimulus to provoke gustatory sweating Patients were required to chew and suck these lemon slices for approximately 2 minutes As noted by Laage Hellman (1957) the Minor test is capable of producing objective documentation of even unappreciable sweating Any sweating whatsoever produces a blue discoloration of the iodine starch mixture which varies in direct proportion to the severity of the sweating Minimal secretion of sweat results in coloration of the openings of the individual sweat glands, as marked by tiny blue punctae More pronounced sweating produces varying confluent areas of blue staining Documentation is accomplished by photography, and the degree of the Frey Syndrome when occurring can be categorized as slight, moderate or severe (Fig 2)

## RESULTS

A composite of all patients studied (Table I) shows that the majority of patients (42/70, 60 %) underwent lateral parotidectomy as treatment for benign tumour In testing patients for incidence of the Frey Syndrome it soon became apparent that pathological diagnosis of the excised parotid gland had no significance in the occurrence of the syndrome Consequently, Tables II and III indicate the incidence and severity of the Frey Syndrome in patients with or without sternomastoid muscle flaps following

Table I *Patients studied in determining the effectiveness of sternomastoid muscle flaps in preventing postoperative occurrence of the Frey Syndrome*

| Years following surgery | No of patients | | Histological diagnosis | |
|---|---|---|---|---|
| | Musc flap | Control group | Chronic parotitis | Benign tumour |
| 1 *Lateral parotidectomy* | | | | |
| 1 | 4 | 1 | 0 | 5 |
| 2 | 5 | 8 | 3 | 10 |
| 3 | 6 | 5 | 0 | 11 |
| 4 | 3 | 8 | 2 | 9 |
| 5 | 3 | 0 | 1 | 2 |
| 6 | 2 | 5 | 2 | 5 |
| 2 *Subtotal/total parotidectomy* | | | | |
| 1 | 2 | 1 | 1 | 2 |
| 2 | 3 | 3 | 1 | 5 |
| 3 | 1 | 1 | 0 | 2 |
| 4 | 3 | 2 | 1 | 4 |
| 5 | 1 | 0 | 0 | 1 |
| 6 | 2 | 1 | 0 | 3 |
| | *35* | *35* | *11* | *59* |

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# THE EFFECTIVENESS OF A STERNOMASTOID MUSCLE FLAP IN PREVENTING POST-PAROTIDECTOMY OCCURRENCE OF THE FREY SYNDROME

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A random sampling of 35 patients with [a] sternomastoid muscle flap as described and [a] control group of 35 patients without such a fla[p] were examined for incidence and severity of th[e] Frey Syndrome following surgery

Of the patients studied, parotid surgery had been performed between 1 and 6 years previously The essential postoperative course of these pa[-]tients had been without significant consequence

Table II *Incidence of Frey Syndrome subsequent to lateral parotidectomy*

| Years following surgery | No of pats | Severity | Muscle flap | | | Controls | | |
|---|---|---|---|---|---|---|---|---|
| | | | Subjective complaints | Minor Test | | Subjective complaints | Minor Test | |
| | | | | Pos | Neg | | Pos | Neg. |
| 1 | 5 | Slight | 0 | 3 | 0 | 0 | 1 | 0 |
| | | Moderate | 1 | 1 | 0 | 0 | 0 | 0 |
| | | Severe | 0 | 0 | 0 | 0 | 0 | 0 |
| 2 | 13 | Slight | 2 | 2 | 0 | 0 | 3 | 0 |
| | | Moderate | 1 | 1 | 0 | 0 | 2 | 0 |
| | | Severe | 2 | 2 | 0 | 1 | 3 | 0 |
| 3 | 11 | Slight | 1 | 4 | 1 | 1 | 5 | 0 |
| | | Moderate | 0 | 1 | 0 | 0 | 0 | 0 |
| | | Severe | 0 | 0 | 0 | 0 | 0 | 0 |
| 4 | 11 | Slight | 0 | 0 | 0 | 2 | 5 | 1 |
| | | Moderate | 1 | 2 | 0 | 1 | 1 | 0 |
| | | Severe | 1 | 1 | 0 | 1 | 1 | 0 |
| 5 | 3 | Slight | 0 | 1 | 0 | 0 | 0 | 0 |
| | | Moderate | 0 | 0 | 0 | 0 | 0 | 0 |
| | | Severe | 2 | 2 | 0 | 0 | 0 | 0 |
| 6 | 7 | Slight | 1 | 1 | 0 | 1 | 3 | 1 |
| | | Moderate | 0 | 1 | 0 | 0 | 1 | 0 |
| | | Severe | 0 | 0 | 0 | 0 | 0 | 0 |
| Totals | 50 | | 12 | 22 | 1 | 7 | 25 | 2 |

Table III *Incidence of Frey Syndrome subsequent to subtotal or total parotidectomy*

| Years following surgery | No of pats | Severity | Muscle flap | | | Controls | | |
|---|---|---|---|---|---|---|---|---|
| | | | Subjective complaints | Minor Test | | Subjective complaints | Minor Test | |
| | | | | Pos | Neg | | Pos | Neg |
| 1 | 3 | Slight | 0 | 1 | 0 | 0 | 0 | 0 |
| | | Moderate | 0 | 0 | 0 | 1 | 1 | 0 |
| | | Severe | 0 | 1 | 0 | 0 | 0 | 0 |
| 2 | 6 | Slight | 0 | 3 | 0 | 0 | 3 | 0 |
| | | Moderate | 0 | 0 | 0 | 0 | 0 | 0 |
| | | Severe | 0 | 0 | 0 | 0 | 0 | 0 |
| 3 | 2 | Slight | 0 | 1 | 0 | 0 | 1 | 0 |
| | | Moderate | 0 | 0 | 0 | 0 | 0 | 0 |
| | | Severe | 0 | 0 | 0 | 0 | 0 | 0 |
| 4 | 5 | Slight | 0 | 1 | 0 | 0 | 0 | 0 |
| | | Moderate | 0 | 1 | 0 | 0 | 2 | 0 |
| | | Severe | 0 | 1 | 0 | 0 | 0 | 0 |
| 5 | 1 | Slight | 0 | 0 | 0 | 0 | 0 | 0 |
| | | Moderate | 1 | 1 | 0 | 0 | 0 | 0 |
| | | Severe | 0 | 0 | 0 | 0 | 0 | 0 |
| 6 | 3 | Slight | 0 | 0 | 0 | 0 | 0 | 0 |
| | | Moderate | 2 | 2 | 0 | 0 | 1 | 0 |
| | | Severe | 0 | 0 | 0 | 0 | 0 | 0 |
| Totals | 20 | | 3 | 12 | 0 | 1 | 8 | 0 |

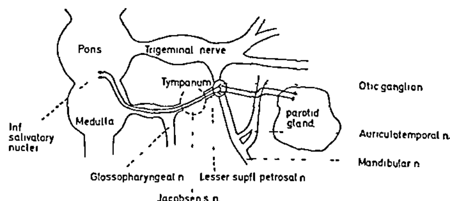



*Fig 3* Normal neural pathway for parotid secretomotor stimulation

## DISCUSSION

The present study attempted to define the effectiveness of a sternomastoid muscle flap in preventing post-parotidectomy occurrence of the Frey Syndrome However, positive documentation for varying degrees of the syndrome was found in almost all patients studied (91 5–100 %), indicating ineffectiveness of the flap in inhibiting gustatory sweating following parotid surgery

Fig 3 shows a schematic representation of the normal neural pathway for parotid secretomotor stimulation Preganglionic parasympathetic fibres initiate in the inferior salivatory nuclei within the lower pons These fibres then pass with the lossopharyngeal nerve from the brainstem, and enter the tympanic cavity through a canaliculus to form the tympanic or Jacobsen's nerve The fibres proceed across the promontory of the middle ear between the round window and Eustachian tube orifice The fibres next become part of the tympanic plexus which overlies the promontory, and exit from the middle ear through the deep or lesser superficial petrosal nerve The preganglionic fibres further pass with this nerve to the otic ganglion, at which site synapses occur with postganglionic neurones These postganglionic fibres leave the ganglion, and continue with the auriculotemporal nerve to the parotid gland As previously noted, disruption of the terminal fibres of the auriculotemporal nerve in some manner produces misdirection of the fibres to skin vessels and sweat glands These endorgans subsequently respond to gustatory stimulation by vasodilation or secretion of sweat

The prevalence of the Frey Syndrome following parotid surgery has previously been we documented by Laage Hellman (1957), and wa corroborated by the present study Under mo circumstances, patients with the syndrome r main clinically asymptomatic or do well wi supportive measures alone This was evide during the present study in that no patient wi subjective or objective evidence for the syndrom considered clinical aid necessary

Varied measures have been employed to mar age the Frey Syndrome in those patients wit significant distress These modalities have in cluded the use of topical or systemic anticho linergic medications, radiotherapy to ablate in volved sweat glands or fibrose surface vessel excision of skin, removal of the otic ganglion section of the auriculotemporal nerve intracra nial division of the glossopharyngeal nerve, a well as intratympanic section of the tympani and chorda tympani nerves (Laage Hellman 1957, Golding Wood, 1962, Hunt et al 1966 Blumenfeld & Friedman, 1967, Smith et al 1970) However, no treatment modality has me with absolute success over prolonged periods of time, and patients invariably learn to tolerate their discomfort

It was intended that a sternomastoid muscle flap used to cover the operative defect following parotidectomy would serve as a barrier between terminal fibrils of the auriculotemporal nerve and skin vessels and sweat glands, thus providing

prophylaxis against possible occurrence of the Frey Syndrome This was not realized by the present study

The sternomastoid muscle flap does have usefulness in minimizing a cosmetic defect resulting from parotid surgery (Jost et al, 1968) However, it does not inhibit gustatory sweating subsequent to surgery, and other means must yet be devised to prevent occurrence of the syndrome following parotid surgery

## ACKNOWLEDGMENTS

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## ZUSAMMENFASSUNG

Unter ca 350 Parotidektomierten der letzten Jahre wurde eine repräsentative Stichprobe von 35 Patienten ausgewählt Bei der I Gruppe wurde ein Schwenklappen des M sternocleidomastoideus gebildet, um das Auftreten des Frey schen Syndroms nach der Parotidektomie zu verhindern Diese Gruppe wurde mit einer Kontrollgruppe von 35 Patienten verglichen, bei denen dieser Schwenklappen nicht benutzt wurde Es zeigt sich dass mit dem Muskelschwenklappen eine bessere kosmetische Deckung des chirurgischen Defektes erzielt werden konnte Das Frey sche Syndrom blieb jedoch unbeeinflusst Es erscheint daher notwendig nach anderen Massnahmen zur Verhinderung des gustatorischen Schwitzens zu suchen

## REFERENCES

Blumenfeld R J & Friedman J E 1967 Intratympanic surgical treatment of Frey s Syndrome *Arch Otolaryng* (Chic) *86* 2

Golding Wood, P H 1962 Tympanic neurectomy *J Laryng 76*, 683

Hunt, W H, Joseph D, Newell, R & Hanna, H H 1966 Gustatory sweating Report of a case treated by tympanic neurectomy *Arch Otolaryng* (Chic) *83*, 260

Jost, G, Legent, F & Baupelot S 1968 Le comblement des dépressions residuelles apres parotidectomie par un lambeau du sterno-cléido-mastoidien Utilisation de l incision de Lotte *Ann Otolaryng* (Par) *85*, 357

Laage Hellman J-E 1957 Gustatory sweating and flushing after conservative parotidectomy *Acta Otolaryng* (Stockh) *48*, 234

Smith, R O Jr, Hemenway, W G Stevens K M & Ratzer, E. R 1970 Jacobson s neurectomy for Frey s Syndrome *Amer J Surg 120*, 478

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# GIANT CELL TUMORS OF BONE IN THE E N T ORGANS

## *Report of Two Cases in the Frontal Sinus*

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(Received March 4, 1973)

*Abstract This is a report on two patients with giant cell* tumors of bone located in the frontal sinus In both patients potential malignancy was suspected The first patient female, had a polyostotic form of the disease *located in the frontal sinus and acromion The second* patient, male, had the cystic form of tumor in the frontal sinus, with involvement of the ethmoidal labyrinth The laboratory findings of both patients showed no abnormalities Both patients were treated surgically and showed no recurrence 2½ years after operation The authors discuss the cases collected from the otolaryngological literature with occurrence in various organs of the E N T region They are very rare in this area and there is therefore little experience of their diagnosis and treatment As the diagnosis of this disease in the E N T organs is made mostly after the operation the post operative biopsy is of utmost importance as to the prognosis Long term observation of patients is imperative Primary irradiation is not recommended

Giant cell tumors of bone are very rare in the E N T organs The diagnosis, prognosis and treatment are of great importance In the last two years two cases of giant cell tumors with an extremely rare localization in the frontal sinus were observed at the E N T clinic of the Faculty of Hygiene in Prague

## CASE REPORTS

### *Case 1*

Female (M No protocol 9176) born 1908, noticed a painless growth in the frontal region within the last 3 months At the time of admission to the clinic a painless swelling of hard consistency, 3 × 3 × 1½ cm in size, was found Other findings in the E N T region were negative, and no lymph nodes were palpable X-ray *(Dr Blaha) revealed a semicircular homogeneous* shadow projecting into the upper part of the frontal sinus (Fig 1) The tumor was excised *together with the periosteum and adjacent* bone It was of chestnut size, filling the upper part of the left frontal cavity The posterior wall of the sinus was eroded up to the thickened dura mater, with a bony defect 2½ × 2½ cm in size The mucous membrane of the sinus was thickened without granulations or secretion

Fig 2 shows the postoperative appearance of the patient The histological report (Dr Stolz) describes a highly vascular tumor invading and destroying the bone, and containing numerous giant cells The tumor showed many areas of fibrosis with apparent collagen formation In some places capillaries predominated, and focal hemorrhages were also noted The bone marrow showed many giant cells of blastomatous type (Fig 3) During a later examination of the whole skeleton X-ray revealed a further focus in the acromion (Fig 4) Laboratory data showed serum calcium 11 4 mg ionised calcium 4 1 mg %, and normal values for phosphorus and for alkaline and acid phosphatases The postoperative course was without complications, and the patient is free from recurrence after 2½ years

### *Case 2*

I H male, born in 1949 (No protocol 6028) Six months prior to admission he observed the left exophthalmus increasing A swelling in the



*Fig 1* X ray showing the tumor of the frontal bone and opacity of the frontal sinus

roof of the orbita was found, occupying the floor of the left frontal sinus (Fig 5) The left eye was depressed downward though without impairment of movement or sight X ray (Dr Blaha) revealed a light diffuse opacity of the left frontal sinus (Fig 6) On tomography the major part of the left frontal sinus was occupied by a tumor of ovoid shape There was a slight opacity in the ethmoidal cells Evidently a part of the roof and of the internal ridge of the left orbit, and the posterior wall of the sinus were destroyed Serum calcium, phosphorus alkaline and acid phosphatases were normal During the operation performed through a Killian skin incision a defect in the inferior wall of the frontal sinus, 2½ × 2½ cm in size, was detected The soft tumor masses prolapsed through the defect and completely filled the widely opened sinus The tumor was dark brown and soft with a solid portion in the lateral recess covered with a film of pus After removal of granulations, a pulsating cyst the size of a hen's egg and containing 15 ml of pink fluid was discovered

The cyst had eroded the posterior wall of the sinus, thus forming a bony defect, 4 × 4 cm in size, and adherent to the dura mater Tumor tissue involved the ethmoidal labyrinth It was exenterated and the tissue was found to have the same appearance as the tumor in the frontal sinus A wide drainage into the nose was performed Normal healing occurred after 2 weeks The patient is now in good health 2½ years after operation (Fig 7)

The histologic finding was as follows The section (Fig 8) shows an invasive osteogenic tumor arising near the periosteum, containing numerous spindle and round cells arranged around branched capillaries Formation of osteoid and mature bone is noticed in some areas Numerous giant cells are found The capillaries are enormously dilated, even to the formation of cavernous spaces The tumor fills the frontal sinus leaving portions of the mucosal lining intact



*Fig 2* The patient after operation

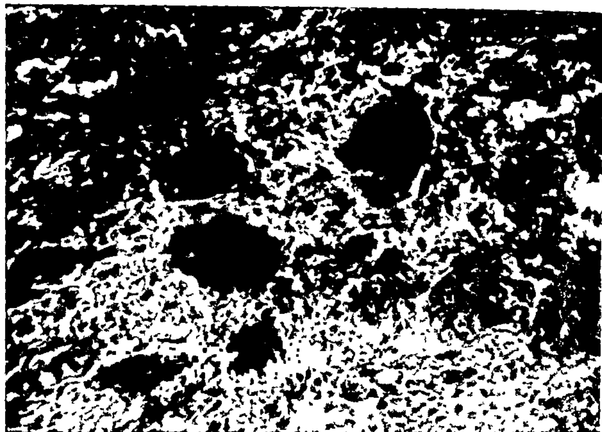


*Fig 3* Histologic picture of tumor tissue Note many giant cells (hematoxylin-eosin × 650)

## DISCUSSION

Jaffe (1964) classifies giant cell tumors into 3 types (1) giant cell tumors of bone, (2) reparative granuloma, (3) giant cell tumors of hyperparathyroidism Zollinger (1968) divides so-called brown tumors into 3 major groups (1) ostitis fibrosa Recklinghausen, (2) juvenile bone cysts (3) osteoclastomas Doerr (1970) calls this group of bone tumors fibromas, which he divides into pure fibromas, solitary bone cysts, and benign giant cell bone tumors Clinically and microscopically there exists considerable confusion as to what constitutes a giant cell tumor Only recent studies using ultramicroscopic methods have brought some clarity in this question These tumors are characterized by the presence of masses of mononuclear cells which are ovoid or spindle-shaped with a small amount of cytoplasm The nuclear membrane is smooth and thin In this mass of small cells, large multinuclear giant cells are embedded They contain up to 80 nuclei, as found by Steiner & Ghosh (1972) in their microscopic studies The paper of Hideya et al (1970) who studied the ultrastructure of these tumors suggests that the giant cells are formed by fusion of round stroma cells These authors also point out that the fine structure of the giant cells is indistinguishable from osteoclasts according to Morton (1956) Stuart called these tumors actually osteoclastomas Steiner & Ghosh (1972) on the basis of ultramicroscopic studies, think that these giant cell tumors originate from undifferentiated connective tissue of bone marrow and giant cells by fusion of mononuclear cells They differ from osteoclasts in that they do not appear to participate in active bone resorption The mononuclear cells rather than giant cells, are the principal cells



Fig 4 Focus of the giant cell tumor in the acromion



Fig 6 X ray showing opacity of the frontal sinus and ethmoidal cells



Fig 5 The patient before operation Note the swelling of the floor of the left frontal sinus



Fig 7 The patient after operation

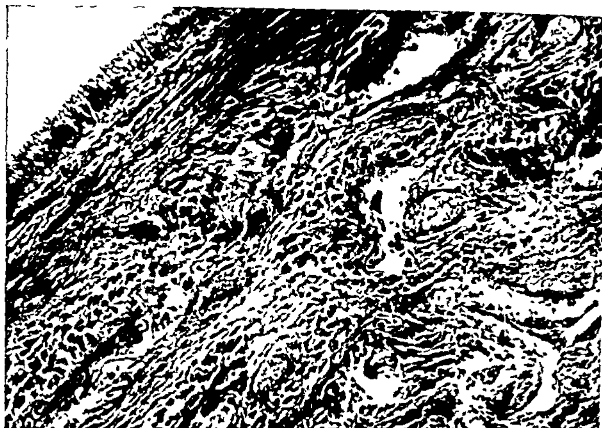


*Fig 8* Histologic picture of the tumor Note the preserved epithelial lining of the frontal sinus (hematoxylin-eosin ×250)

in this tumor In many of them areas of collagen deposits and spicules of bone are noted According to Jaffe the hemorrhage described in many of these tumors is a differential point When present the brown tumor of hyperparathyroidism should be suspected Crowding of the stromal cells and a decrease in the number of giant cells, as well as an increase in the number of mitoses is considered a sign of greater aggressive potential According to Murphy & Ackerman (1956) approximately 10% of all giant cell tumors are malignant at the time of first examination

As far as the origin of giant cell tumors is concerned, Geschickter & Copeland (1936) suggest that they arise from abnormal hyperplasia of osteoclasts beside areas of enchondral ossification On the other hand Jaffe (1964) thinks they originate from undifferentiated supporting connective tissue of the bone marrow

As with many other bone tumors trauma or congenital origins have been suspected as etiologic agents, though without proof

*Incidence*

Giant cell tumors occur mostly in long bones of the skeleton The frequency of their occurrence in the maxillary cavity, mandible and alveolar process is approximately 9% (Švejda, 1968) Hlavaček (1931) published an earlier report of 3 patients with localization in the mandible These tumors are rare in the E N T organs They occur mostly in the maxillary cavity (Kutvirt, 1926, Geschickter & Copeland, 1936 Mela & Mongini, 1966) Localization in the frontal bone has been reported by Horníček (1948) and by Gignoux et al (1957) Nasal bones were involved in the case of Sgouras (1971) The sphenoidal sinus may be site of

giant cell tumor as well, as reported by Geissinger et al (1970) and by Gary et al (1970) Emley (1971) collected 14 cases in this site from the world literature I have not found any case in the literature describing giant cell tumor with a large cyst in the frontal sinus, as was the case in one of our patients The region of the temporal bones is also sometimes the site of occurrence of these tumors but very rarely The squamous temporal bone and mastoid process were involved in the cases of Kutvirt (1926), Horníček (1948), Ježek (1951) and Sakamoto (1970) The middle ear was involved in the cases of Rosenwasser (1969) and Gignoux (1970) Hall (1972) reported the very rare occurrence of a giant cell tumor in the larynx

The course of the disease is slow and without appreciable pain if the tumor is localized in the E N T organs Malignant variants are rarely observed but local recurrences are frequent

*Diagnosis*

Diagnosis rests on physical and laboratory examination, X ray, and careful clinical survey If sufficient tissue is available, biopsy is most important for diagnosis (Morton 1956) Diagnosis from biopsy rests on a careful evaluation of both the stroma and the giant cells Many bony lesions have giant cells The lesion most likely to be confused with giant cell tumors such as the so-called brown tumor of hyperparathyroidism In the latter lesion giant cells are less numerous unevenly distributed and often surrounded by areas of haemorrhage Serum calcium phosphorus and alkaline and acid phosphatase determinations are invaluable aids to making the correct diagnosis While making the diagnosis one has to take into consideration the fact that giant cells are present in many other lesions of bone in benign chondroblastomas non osteogenic fibromas aneurysmal bone cysts simple bone cysts with cellular lining reparative granuloma, lesions of hyperparathyroidism and osteogenic sarcoma All these must be considered in the differential diagnosis, as stated by Dahling et al (1962)

*Treatment*

Treatment consists mainly of surgical procedure, i e excochleation and if the tumor is strictly delimited, in complete extirpation of the tumor, together with adjacent bone Primary irradiation is contra indicated since the tumor may undergo malignant changes As histological examination cannot altogether exclude malignancy, long term observation of the patient is necessary


## RESUME

Rapport sur deux malades atteints de tumeurs a cellules geantes localisees dans le sinus frontal La premiere malad etait atteinte d une polyostotic form avec la localisation de la tumeur dans le sinus frontal et acromion Le deuxieme patient homme avait une cystic form de la tumeur dans le sinus frontal avec l envahissement du labyrinth ethmoidal Le bilan humoral de ces malades etait normal Les malades etaient traite chirurgicallement et sont 2½ annees sans recidive

## ZUSAMMENFASSUNG

Ein Bericht uber zwei Kranken die mit gigant zellen Geschwulsten der Stirnhohle betroffen waren Die erste Patientin wies eine polyostotische Form aus Die Geschwulst wurde in der Stirnhohle und Acromion lokalisiert Der zweite Kranke hatte eine zystische Form der Geschwulst in der Stirnhohle Die geschwulstartigen Veranderungen breiteten sich auch im ethmoidalen Labyrinth aus Die laboratorischen humoralen Befunden wiesen bei beiden Kranken keine Abnormalitat aus Beide wurden chirurgisch behandelt und sind 2½ Jahre ohne Rezidive


## REFERENCES


Dahling D C Cupps R E & Johnson E W 1962 Giant cell tumor A study of 195 cases *Cancer 15* 1061

Doerr U 1970 *Spezielle Pathologische Anatomie* Vol 2 Springer Verlag

Emley W 1971 Giant cell tumor of the sphenoid bone *Arch Otolaryng* (Chic ) *94* 369

Gary L Townsend L & De Santo L W 1970 Malignant change in sphenoid sinus fibrous dysplasia *Arch Otolaryng* (Chic ) *9?* 267

Geissinger J Siqueira E B & Ross E R 1970 Giant cell tumors of the sphenoid bone *J Neurosurg 32* 665

Geschickter Ch F & Copeland M M 1936 *Tumors of bone* Lippincott Philadelphia

Gignoux M 1970 Dysplasie fibreuse de l oreille moyenne *Cah Otolaryng 5* 875

Gignoux M Paufique L & Carre I 1957 Les tumeures osteofibreuses rares du sinus frontal et de la voute orbitaire *Ann Otolaryng* (Par ) *74* 348

Hall, J J 1972 Giant cell tumor of the larynx *J Lar Otol 86*, 371

Hideya, H, Friedmann, B & Mack, R P 1970 Ultrastructure and histogenesis of giant cell tumor of bone *Cancer 25*, 1408

Hlaváček, V 1931 Cases of localized ostitis fibrosa of the inferior maxillary bone *Otolaryng Slavica 3*, 7

— 1948 Fibrosni dysplasie kosti lebnich *Čas Lek Cesk 87*, 521

Horníček, V 1948 Fibrosní dysplasie kosti lebnick *Čas Lek Čes 87*, 18, 521

Jaffe, H L 1964 Tumors and tumorous conditions of the bone and joints Lea & Febiger, Philadelphia

Ježek, K 1951 Pripad fibrosni dysplasie kostni vyčnělku soscovitého *Čas Lek Česk 90*, 1386

Kutvirt, O 1926 Ostitis fibrosa Recklinghausen maxillae superioris *Zráva o pátem výrocním sjezdu čsl otol společnosti*

Mela, F & Mongini, F 1966 Posttherapeutic examination of 27 cases of giant cell neoformation of the maxillary antrum bone *Minerva Stomatol 15*, 732

Morton, J J 1956 Giant cell tumor of bone *Cancer 9*, 1012

Murphy, W R & Ackerman, L V 1956 Benign and malignant giant cell tumors of bone *Cancer 9*, 317

Rosenwasser, H 1969 Giant cell tumor involving the middle ear *Arch Otolaryng* (Chic.) *90*, 726

Sakamoto, S 1970 *Exc Med XI* Ref No 3979

Sgouras, N 1971 Giant cell tumor of nasal bones *Plast Reconstr Surg 48*, 184

Steiner, G C & Ghosh, L 1972 Ultrastructure of giant cell tumor of bone *Hum Pathol 3*, 569

Švejda, J 1968 K problematice obrovskobeněčných nádoru čelisti *Čs stomatol 2*, 85

Zollinger, H 1968 *Pathologische Anatomie* Thieme, Stuttgart

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# THE VASCULAR ANATOMY OF THE RHESUS MONKEY COCHLEA

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*Abstract* The vascular pattern of the Rhesus monkey cochlea was demonstrated by the aid of contrast injection The capillary areas are very similar to the human, rabbit, and guinea pig cochlea One significant exception is the unfrequent occurrence of a vessel under the tunnel of Corti Further, the capillary areas of the external wall at the basal end of the cochlea are more well formed than in the other mammals The course and ramification of the veins in the modiolus is more irregular than in the rabbit and guinea pig cochlea and more similar to the human cochlea The irregular occurrence of the vessel under the outer hair cells indicates that the Rhesus monkey is a particularly interesting animal for cochlear research

The cochlear vascular anatomy of those mammals which are most commonly assessed by experimental otologic research is in general well known (see Axelsson, 1968) However, the vascular anatomy of the Rhesus monkey cochlea has not been reported and in general there are only sparse reports concerning the cochlear vascular anatomy of any monkeys The importance of investigating the Rhesus monkey is based primarily upon the increasing use of this mammal for experimental cochlear research and the presumed similarity between monkey and man in regard to both cochlear anatomy and function

The earliest description of the vascular anatomy in the monkey (Macacus) was given by Nabeya (1923) The most remarkable findings in the Macacus were one spiral vessel under the tunnel of Corti and two zones of capillary network one in the spiral limbus and one in the tympanic lip There were individual variations in the gross venous drainage from the cochlea between animals Nabeya concludes that all capillaries of the labyrinth of the monkey closely resemble those of the human ear

Hawkins & Johnsson (1968) reported a large outer spiral vessel in the Rhesus monkey fetus In a later fetal stage an inner spiral vessel could be observed simultaneously to the outer spiral vessel These authors demonstrated two spiral vessels in the spiral lamina of the squirrel monkey Johnsson (1972) reports an incomplete outer spiral vessel or none at all, the vessel being best developed in the basal turn in different monkeys, i e pigtail, crab-eating, stump-tail Rhesus and squirrel monkey

The purpose of this study is to extend the sparse current knowledge of the vasculature of the monkey cochlea

## MATERIAL AND METHODS

Six young, healthy Rhesus monkeys were used for this study The cochlear vasculature was assessed following cochlear perfusion with contrast media The technique has previously been described in detail (Axelsson, 1968, 1971, 1972) Briefly, the vascular system of the animal is perfused with Ringer's solution and subsequently with Prussian blue solution under static pressure The temporal bones are fixed, decalcified neutralized, washed, dehydrated, and stored in glycerin The examination of the cochlea is

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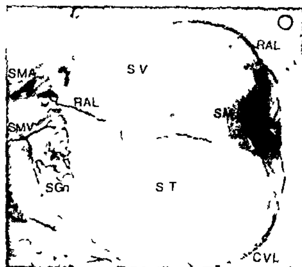



*Fig 1* Basal turn of the Rhesus monkey cochlea, apico basal section The spiral modiolar artery (*SMA*) is situated at the level of the scala vestibuli (*SV*) and supplies the capillary areas in the external wall and the spiral lamina by radiating arterioles (*RAL*) The spiral modiolar vein (*SMV*) varies in course and is in this animal situated at the level of the spiral ganglion (*SGn*) SMV receives collecting venules (*CVL*) from all capillary areas *SM* = scala media, *ST* = scala tympani

done in the stereomicroscope and the photographic documentation made with the aid of the photomicroscope

## RESULTS

### *Modiolus*

*the modiolus the following regularly occurring* vessels can be identified in the Rhesus monkey

- Spiral modiolar artery
- Spiral modiolar vein
- Radiating arterioles
- Collecting venules
- Capillaries in the spiral ganglion
- Capillaries in the acoustic nerve
- Capillaries in the modiolus wall

The Rhesus monkey cochlea has three turns The *spiral modiolar artery* (SMA) derives from the common cochlear artery in the basal turn, about a fourth of a turn from the basal end of the cochlea The common cochlear artery here divides into the vestibulo-cochlear artery and the spiral modiolar artery The spiral modiolar artery takes a serpentine course around the modiolus up to the cochlear apex and is situated, as in other mammals, at the level of the scala vestibuli of the modiolus (Fig 1) Numerous *radiating arterioles* (RAL) branch off from the spiral modiolar artery to supply the capillary areas of the external wall, the spiral lamina, as well as the spiral ganglion, VIII nerve, and the wall of the modiolus

The *spiral modiolar vein* (SMV) is situated in the lower medial aspect of the scala tympani in the two apical turns In the basal turn there appears to be variations in the course of the spiral modiolar vein The vein sometimes appears in the lower medial aspect of the scala tympani but sometimes at the level of the scala vestibuli (Fig 1) In the latter case it receives large venous branches by a smaller vein of the lower medial aspect of the scala tympani The *collecting venules* (CVL) derive from capillaries in the external wall, the spiral lamina, the spiral ganglion and the modiolus wall

Similar to the other mammals, the VIIIth nerve (Fig 2) and the spiral ganglion (Fig 3) are supplied by many delicate capillaries without any regularly occurring arrangement *In the* basal turn, the spiral modiolar vein has connections with delicate vessels in the bone surrounding the cochlea The spiral modiolar vein empties *in the region of the round window into the vein* of the cochlear aqueduct The vein of the



*Fig 2* Capillaries of the acoustic nerve Delicate capillaries run in different directions without any significant pattern

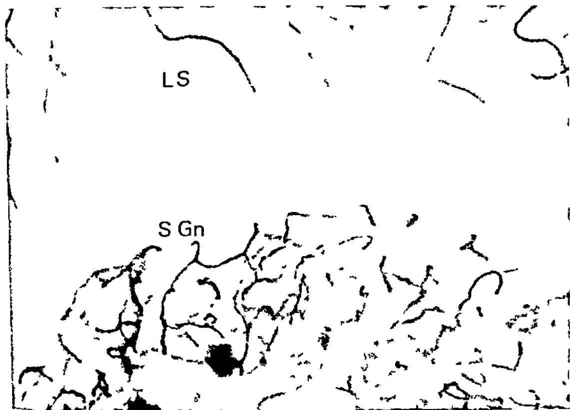



*Fig 3* Spiral ganglion basal turn transverse section Delicate capillaries form a dense vascular net in the spiral ganglion (*SGn*) without any specific arrangement *LS* lamina spiralis

cochlear aqueduct receives the vein of the round window and sometimes a separate vein of the scala tympani

*Spiral Lamina*

In the Rhesus monkey the following vascular structures can be identified regularly

- Radiating arterioles
- Collecting venules
- The vessel of the tympanic lip
- The limbus vessels

*Radiating arterioles* (RAL) (Figs 1 4 6) leave the spiral modiolar artery and have a coiled course centrally in the spiral lamina but straighten out more peripherally They supply the capillary areas in the spiral limbus and the tympanic lip in a more or less T or Y formed manner Each radiating arteriole supplies a fairly small segment of the peripheral vascular margins in the tympanic lip and spiral limbus The appearance of the radiating arterioles is very similar to that of the rabbit and human cochlea The *collecting venules* (CVL) (Figs 1 4 6) drain the spirally running capillary vessels at fairly right angles in a T or Y formed manner similarly to the radiating arterioles and run radially and centripetally over the spiral lamina to empty into the spiral modiolar vein Often branches of the collecting venules anastomose centrally in the spiral lamina The collecting venules also appear similar to those of the human rabbit and guinea pig cochlea In all three turns the radiating arterioles and collecting venules also anastomose central to the vessel of the tympanic lip and form vascular arcades here (Figs 4 6)

Infrequently small segments of the *vessel of the basilar membrane* (VSBM vas spirale) can be identified in the second and third turns of the monkey and very infrequently in the basal turn

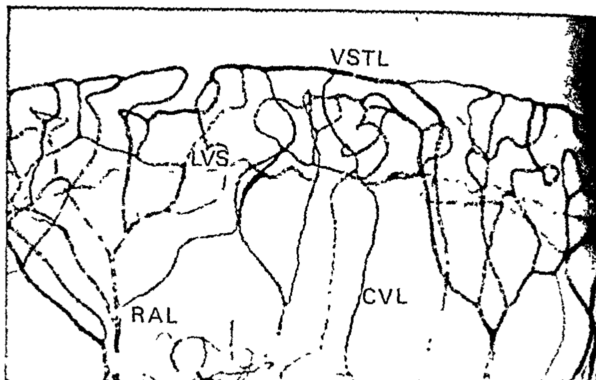



*Fig 4* Spiral lamina, basal turn, transverse section Vascular arcades form a spiral vascular margin the vessel of the tympanic lip (*VSTL*) There is no regularly occurring vessel under the outer hair cells Radiating arterioles (*RAL*) supply the capillary arcades (*VSTL*) and the limbus vessels (*LVS*) The vascular arcades are drained by collecting venules (*CVL*)

(Fig 5) This vessel does not occur regularly in the rabbit, but is found consistently in the guinea pig and human

The *vessel of the tympanic lip* (VSTL) is situated at the level of the inner hair cells in the tympanic lip and constitutes the most peripheral spirally running capillary border of the spiral lamina (Figs 4 5, 6) It is alternately supplied and drained by radiating arterioles and collecting venules Often capillary arcades are seen centrally to the vessel of the tympanic lip particularly in the basal turn (Fig 4) These arcades may take a spiral course for short distances almost forming a spiral capillary border centrally and parallel to the vessel of the tympanic lip

*External Wall*

*Scala vestibuli*

The following regularly occurring vessels could be demonstrated in the external wall

Radiating arterioles
Collecting venules
Sparse capillary loops apical to the attachment of the vestibular membrane
The vessel at the vestibular membrane

The *radiating arterioles* (RAL) derive from the spiral modiolar artery and first run a serpentine centrifugal course over the scala vestibuli and then straighten more peripherally (Fig 8) The radiating arterioles supply all capillary areas in the external wall The *collecting venules* (CVL) are more delicate and have a more irregular course than the radiating arterioles (Fig 8) They drain the capillaries and the vessel at the vestibular membrane The collecting venules run both in an apical direction to the spiral modiolar vein of the next apical turn and in a basal direction to collecting venules from the scala tympani of the same turn externally to the stria vascularis. The *vessel at the vestibular membrane* (VSVM)

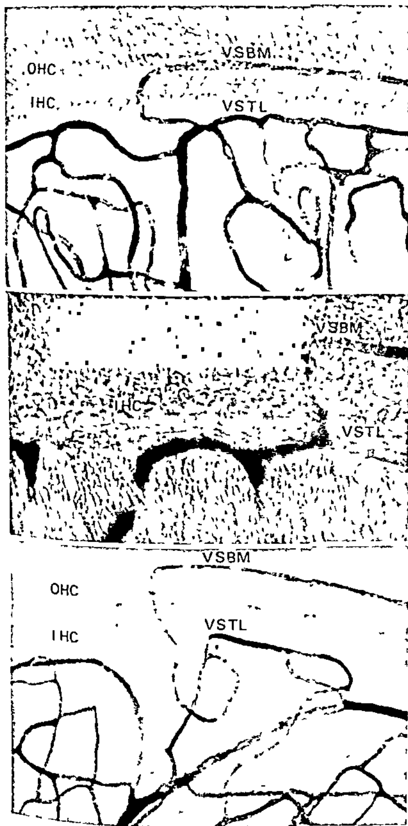
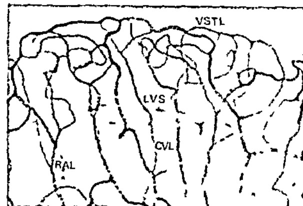



*Fig 5* Spiral lamina, second turn, transverse section An irregular vascular margin is formed under the tunnel of Corti corresponding to the vessel of the basilar membrane (*VSBM*) *VSTL* = vessel of the tympanic lip *OCH* = outer hair cells *IHC* = inner hair cells.



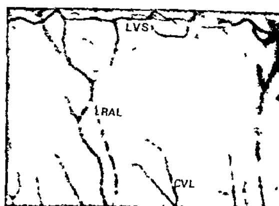




*Fig 6* Spiral lamina second turn transverse section The peripheral vascular margin the vessel of the tympanic lip (*VSTL*), is less straight than in the basal turn There are many anastomoses between the radiating arterioles (*RAL*) and the collecting venules (*CVL*) central to the VSTL *LVS* = limbus vessels

*Fig 7* Spiral limbus, basal turn, transverse sectio Radiating arterioles (*RAL*) supply and collecting venule (*CVL*) drain the capillary vascular margin of the spir limbus, the limbus vessels (*LVS*)



*Fig 8* External wall basal turn apicobasal section Large radiating arterioles (*RAL*) supply and delicate collecting venules (*CVL*) drain the vessels in the scala vestibuli A basal vascular border is formed by the vessel at the vestibular membrane (*VSVM*) The stria vascularis (*SVS*) and the vessel of the spiral prominence (*VSSP*) are equally supplied by the RAL The collecting venules (*CVL*) drain all vessels in the external wall and form a spirally running vessel immediately basal to the attachment of the basilar membrane the venules of the basilar membrane (*VLBM*)

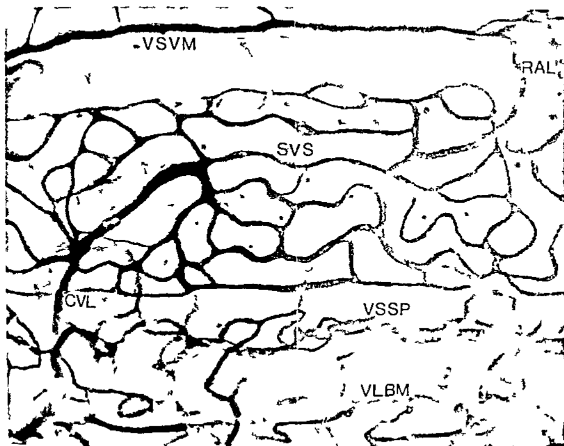



Fig 9 External wall basal turn apicobasal section The vessel at the vestibular membrane (*VSVM*) is well formed. The stria vascularis (*SVS*) forms many capillary loops The vessel of the spiral prominence (*VSSP*) is made up of spirally running segments of capillaries The venules at the basilar membrane (*VLBM*) are equally formed by spirally running portions of the collecting venules (*CVL*) *RAL* radiating arterioles

is clearly evident as a spiral vascular border above the vestibular membrane in all three turns (Figs 8 9) The vessel at the vestibular membrane constitutes the basal border of the capillary loops in the scala vestibuli The capillary loops of the scala vestibuli are formed by the sparse capillary connections of the finest branches from the radiating arterioles and collecting venules They are too sparse to deserve the designation of a capillary net There is no spirally running vessel of the scala vestibuli as it appears in the guinea pig

*Scala media*

The following vascular structures can regularly be demonstrated in the scala media

Stria vascularis
The vessel of the spiral prominence
Arteriovenous anastomoses

The *stria vascularis* (SVS) constitutes the most prominent vascular structure in the scala media containing a capillary net which includes a breadth of six to eight loops in the basal turn and one single loop in the third turn (Figs 8 9)

Stria vascularis is very similar to the same structure in the guinea pig rabbit and man The stria vascularis is supplied by large but quite sparse radiating arterioles from the scala vestibuli and drained by equally large and sparse collecting venules derived from the scala tympani

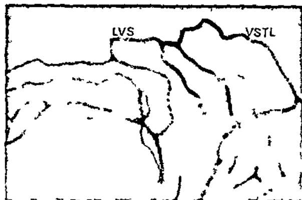



*Fig 10* Spiral lamina apical end The vascular pattern is much simplified and the vasculature terminates in simple vascular arcades *VSTL* = vessel of the tympanic lip *LVS* = limbus vessels Helicotrema to the lower left

(Fig 8) There are no other vascular connections to the stria vascularis

The *vessel of the spiral prominence* (VSSP) appears more irregular than in the guinea pig and is more similar to the corresponding vessel in man (Figs 8, 9) It is formed from short segments of spirally running capillary vessels which are supplied by large and quite frequent radiating arterioles and drained by many collecting venules There are no other vascular connections to the spiral prominence

*Arteriovenous anastomoses* (AVAS) run external to the stria vascularis and the vessel of the spiral prominence These vessels connect branches from the radiating arterioles in the scala vestibuli with collecting venules in the scala tympani (Figs 8, 9) Similar to other mammals referred to above, the number of arteriovenous anastomoses is largest in the basal turn and least in the apical turn

*Scala tympani*

The following vascular structures can regularly be demonstrated in the scala tympani

Collecting venules
The venules at the basilar membrane

The *collecting venules* (CVL) are most numerous in the basal turn and decrease in frequency apically (Figs 8, 9) They drain all the capillary areas in the external wall and anastomose with radiating arterioles from the scala vestibuli externally to the stria vascularis Similar to the other mammals investigated, the collecting venules seem to "grip" around the attachment of the basilar membrane in an omega formed manner The collecting venules run a centripetal course over the scala tympani and often merge to form larger collecting venules basally in the scala tympani The larger collecting venules empty into the spiral modiolar vein

The *venules at the basilar membrane* (VLBM) are formed by the spirally running parts of the *collecting venules immediately below the attachment of the basilar membrane* in the external wall (Figs 8, 9) In this way, a seemingly spirally running vessel is formed

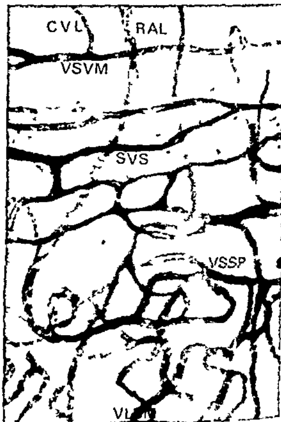



*Fig 11* External wall second turn apicobasal section The stria vascularis (*SVS*) shows an increasing narrowing towards the apex The vascular pattern is otherwise maintained *RAL* radiating arteriole *CVL* = collecting venule *VSVM* vessel at the vestibular membrane *VSSP* = vessel of the spiral prominence *VLBM* = venules at the basilar membrane

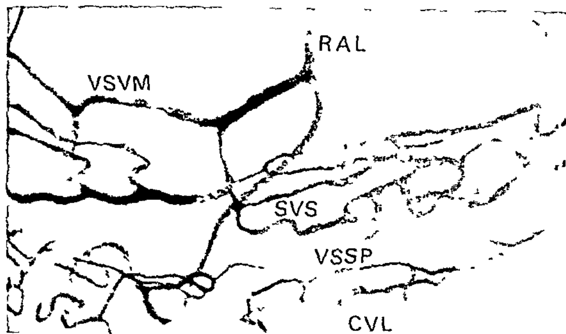



*Fig 12* External wall apical end apicobasal section The vascular pattern is much simplified Many of the regularly occurring vessels can still be identified *RAL* = radiating arteriole *VSVM* vessel at the vestibular membrane *SVS* stria vascularis *VSSP* vessel of the spiral prominence *CVL* collecting venules

*Apical end*

Similar to man rabbit, and guinea pig, the apical end is characterized by a seemingly pronounced sparseness of the vasculature In the spiral lamina (Fig 10) the vessel of the tympanic lip can be identified up to the apical end, and there is often a vessel of the basilar membrane present in the most apical part The limbus vessels run a more uneven course than more basally In the external wall (Figs 11, 12) the number of arteriovenous anastomoses decrease The radiating arterioles particularly supply the stria vascularis and the vessel of the spiral prominence which are the most prominent capillary structures at the level of the apex The vessel at the vestibular membrane runs straight spirally and is well maintained up to the apical end

*Basal end*

In the basal end, the radiating arterioles (RAL) deriving from the vestibulo-cochlear artery run an oblique course towards the scala vestibuli in the region of the oval window (Fig 13) When the vestibulo-cochlear artery arrives in the vestibulum one or two arterial branches (RAL) run around the extreme basal end of the spiral lamina and return 'upward to merge with radiating arterioles also running obliquely over the scala vestibuli from the apical cochlear side of the oval window A capillary net is formed in the scala vestibuli where the branches of the two radiating arterioles merge from opposite directions In the scala tympani the collecting venules (CVL) drain into the vein of the round window which runs around the apical aspect of the round window, similar to the other mammals investigated The capillary vessels in the spiral lamina and the external wall are well formed to the extreme basal end (Fig 13)

## DISCUSSION

The Rhesus monkey has recently become a frequently used mammal for cochlear research Consequently, basic anatomical information concerning all parts of the cochlea is needed

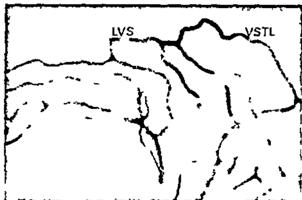



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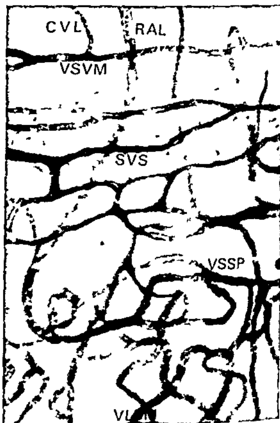



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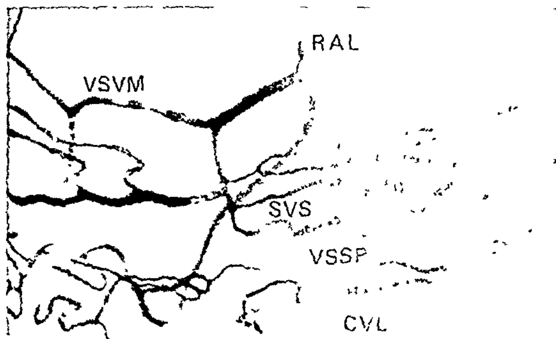



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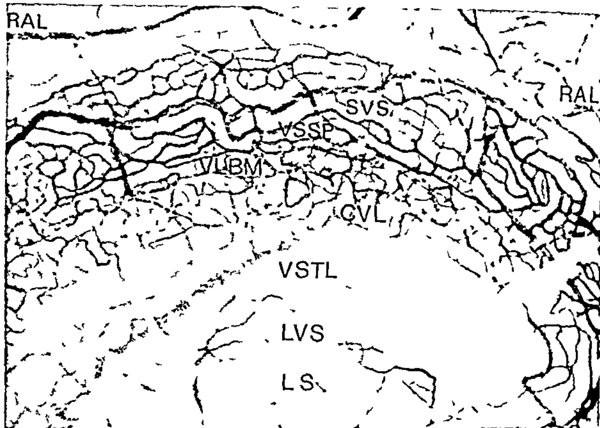



*Fig 13* Basal end In the region between the oval window and the round window the radiating arterioles (*RAL*) coming from the cochlear side (*left*) merge with radiating arterioles coming from the vestibulum (*right*) The vascular tributaries of the external wall are well formed here *SVS* stria vascularis *VSSP* = vessel of the spiral prominence *VLBM* = venules at the basilar membrane *CVL* – collecting venules In the lower part the sparse vasculature of the spiral lamina (*LS*) can be seen *VSTL* = vessel of the tympanic lip *LVS* = limbus vessels

From the technical viewpoint, the Rhesus monkey is easily injected and yields consistant good results at least with the present small numbers (six animals twelve cochleas) The removal of the temporal bones after injection requires more time than in either the guinea pig or the rabbit and amounts to three to six minutes The subsequent technical steps were easily achieved and all parts of the cochlea can be demonstrated in surface specimen preparation, just as easily as in the guinea pig or rabbit cochlea

The nomenclature adopted on the cochlear vessels is based on the relation of vascular structures to other anatomical structures or on the course of the vessels in the cochlea The nomenclature consequently is not based on histological examinations of the vascular wall which may raise disagreements in naming vessels An arteriole, i e is here looked upon as a vessel connecting a large supplying artery with the terminal vascular bed where the vessels are termed capillaries regardless of their morphological structure Equally, a collecting venule is considered as a vascular structure connecting the capillaries with a vein etc

In general the vascular anatomy of the Rhesus monkey cochlea is very similar to the human guinea pig and rabbit (Axelsson 1968 1971 Axelsson & Lind, 1973) The main characteristics of the vascular anatomy in the Rhesus monkey are

The arterial system including larger arteries as well as radiating arterioles is similar to the other mammalian species investigated so far (man, rabbit, guinea pig)

The venous system is more irregular than in the guinea pig and rabbit and is more similar to the human cochlea in showing variations particularly in the basal turn

The collecting venules in the external wall merge to large collecting veins more peripheral in the scala tympani than in the human, rabbit or guinea pig cochlea

The vessel of the basilar membrane only occurs irregularly and most often in the third and second turn In this respect the Rhesus monkey cochlea is similar to the rabbit cochlea The spirally running capillary vascular border in the tympanic lip and in the spiral limbus is similar to the other mammals investigated

In the external wall there is only a very sparse capillary net in the scala vestibuli and no spirally running vessel of the scala vestibuli as in the guinea pig The capillary areas in the external wall otherwise are similar to the human, rabbit and guinea pig cochlea

At the basal end (the hook region) all vascular tributaries in the external wall are wellformed and more so than in the human, rabbit and guinea pig cochleas

As reviewed in the introduction, very sparse information is available concerning the vasculature of the monkey cochlea The main point of controversy seems to be the number of spiral vessels in the spiral lamina In the present investigation, the principal finding was one spiral capillary vessel of the tympanic lip and an unfrequent occurrence of the vessel of the basilar membrane When this vessel could be demonstrated it was most frequently found in the second and third turn As stated from several previous investigations the vessel of the basilar membrane might be of its greatest importance during fetal life with a subsequent involution (Hawkins & Johnsson, 1968 Hilding 1969, Johnsson, 1972 Johnsson & Hawkins 1972) Thus also suggests that the importance of this vessel for the oxygen supply to the organ of Corti may be less pronounced in postfetal life than suggested by many authors This is also suggested by the finding that several species of mammals lack this vessel, i e rabbit (Retzius, 1884 Nabeya, 1923, Axelsson & Lind, 1973) cat (Smith 1954) and mink (Hilding et al , 1967)

The venous system of the modiolus appears more irregular than in the guinea pig and rabbit and consequently is more similar to the complicated and largely individually varied appearance of the human cochlea The sparse connections between the veins of the modiolus and the surrounding bone vessels are not of the size or frequency to indicate any function of significant importance The favorable outcome of the injections and the inconsistency of a capillary vessel under the outer hair cells make the Rhesus monkey a most interesting experimental animal for cochlear research

## ACKNOWLEDGMENT

Assoc Prof Lars-Goran Johnsson Kresge Hearing Research Institute is gratefully acknowledged for his support and encouragement to this investigation

## ZUSAMMENFASSUNG

Das Gefassystem der Rhesusaffen Cochlea wurde mit Hilfe von Kontrastmittelinjektion dargestellt Die Kapillarnetze sind denen des Menschen, des Kaninchens und Meerschweinchens sehr ahnlich Ein Hauptunterschied ist ein unregelmassiges Vorhandensein des Gefasses unter dem Cortischen Tunnel Die Kapillarnetze der Aussenwand am basalen Cochlea Ende haben eine grossere Gefassversorgung als beim Menschen Kaninchen und Meerschweinchen Der Verlauf und die Verzweigung der Venen im Modiolus sind unregelmassig und ahneln der menschlichen Cochlea mehr als der des Kaninchens und Meerschweinchens Das unregelmassige Vorhandensein des Gefasses unter dem Cortischen Tunnel und der ausseren Haarzellen macht die Rhesusaffencochlea besonders interessant fur die Gehorforschung

## REFERENCES

Axelsson A 1968 The vascular anatomy of the cochlea in the guinea pig and in man *Acta Otolaryng* (Stockh ), Suppl *243*

— 1971 The cochlear blood vessels in guinea pigs of different ages *Acta Otolaryng* (Stockh ) *72* 172

— 1972 The demonstration of the cochlear vessels in the guinea pig by contrast injection *J Laryng 86* 121

Axelsson A & Ernstson, S 1972. Cochlear vascular anatomy in a strain of the waltzing guinea pig *Acta Otolaryng* (Stockh ) *74* 172

Axelsson, A & Lind A 1973 The capillary areas in the rabbit cochlea *Acta Otolaryng* (Stockh ) *76*, 254
Hawkins, J E , Jr & Johnsson, L G 1968 Light microscopic observations of the inner ear in man and monkey *Ann Otol 77*, 608
Hilding D A 1969 Electron microscopy of the developing hearing organ *Laryngoscope 79*, 1691
Hilding D A , Sugiura, A & Nakai, Y 1967 Deaf white mink electronmicroscopic study of the inner ear *Ann Otol 76*, 647
Johnsson, L G 1972 Cochlear blood vessel pattern in the human fetus and postnatal vascular involution *Ann Otol 81*, 22
Johnsson, L G & Hawkins, J E Jr 1972 Symposium on basic ear research II Strial atrophy in clinical and experimental deafness *Laryngoscope 82*, 1105
— 1972 Vascular changes in the human inner ear associated with aging *Ann Otol 81*, 364
Nabeya, D 1923 A study in the comparative anatomy of the blood vascular system of the internal ear in Mammalia and in Homo (Japanese) *Acta School Med Univ Imp Kyoto 6*, 1
Retzius, G 1884 *Das Gehororgan der Wirbeltiere* II p 269 Samson & Wallin, Stockholm
Smith, C A 1954 Capillary areas of the membranous labyrinth *Ann Otol 63*, 435

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# DISTRIBUTION OF THE CROSSED OLIVO-COCHLEAR BUNDLE TERMINALS IN THE SQUIRREL MONKEY COCHLEA

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*Abstract* Distribution of the crossed olivo-cochlear bundle terminals in the squirrel monkey cochlea was studied electron microscopically after transection of the bundle at the floor of the fourth ventricle About 70% of the inner spiral bundle belongs to the crossed olivo-cochlear bundle at the lower basal turns About 90% of all efferent endings to the outer hair cells belong to the crossed olivo-cochlear bundle within the lower basal turns The innervation percentage of the crossed olivo-cochlear bundle is relatively reduced within the upper turns. After studying the serial transverse sections of the brain stem, it was found that all of the midline sections extended well beyond the edge of the facial genu both rostrally and caudally

The olivo-cochlear bundle (OCB), which is a part of the auditory efferent system from the brain stem to the organ of Corti, was first described by Rasmussen in the cat Since that time, the course of this bundle and distribution patterns of peripheral terminals within the organ of Corti have been studied in many different animal species The histochemical approach using acetylcholinesterase staining, etc, the electron microscopic investigation of peripheral terminals, and the nerve degeneration studies by Nauta staining were commonly used (Rasmussen, 1953, Schuknecht et al, 1959, Hilding & Wersäll, 1962, Gacek et al, 1965, Terayama & Yamamoto, 1971, Spoendlin, 1967) The distribution patterns of the OCB peripheral terminals within the organ of Corti were electron microscopically studied by Kimura & Wersäll (1962) in the guinea pig, by Iurato (1962) in the rat, by Smith & Rasmussen (1963, 1965) and Iurato et al (1968) in the chinchilla, and by Spoendlin & Gacek (1963) in the cat

By utilizing avoidance behavioral conditioning, the authors have studied the psychophysiological contribution of the crossed OCB system to various aspects of systemic auditory function (Igarashi et al, 1972) However, as the OCB peripheral distribution pattern varies slightly among different animal species, and as the psychobiological importance of this OCB system should differ from species to species, there is reason to extend the investigation to the experimental primate

The main purpose of the present investigation is to set up the first step in utilizing primate subjects for investigation In the first step of this study, the distribution pattern of the crossed OCB terminals within the organ of Corti was electron microscopically investigated by utilizing normal adult squirrel monkeys

## METHODS

Ten young adult squirrel monkeys, weighing 500–700 grams, free from any middle ear disorders (based upon otoscopic observation and confirmation at the time of removal of electron

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microscopic specimens) were used as the experimental animals in the present investigation

The intraperitoneal injection of sodium pentobarbital (30 mg/kg) was used for the animal anesthesia The occipital midline incision was made and the bony calvarium opened to expose the posterior portion of the cerebellum and brain stem The cerebellum (mostly the area of the uvula) was gently elevated by a non rigid metal retractor By using a fine pick, the crossed OCB was sectioned at the region of the facial genu in the floor of the fourth ventricle The midline incision was intentionally extended rostrally and caudally at least 1–2 mm beyond the edge of the facial genu During this procedure, care was taken not to retract the cerebellum too much or for too long All bleeding spots were controlled, the area covered by a thin piece of gelfoam, and the wound closed

The animals were sacrificed 14 days after surgery, except for one subject after 7 days, one after 17 days and two after 24 days The monkeys were deeply anesthetized and the inner ear was approached retroauricularly The perilymphatic scalae were perfused with 2 % osmium tetroxide solution After decapitation, the temporal bones were removed and further immersed in the identical fixative and refrigerated for 2 hours Then the specimens were dehydrated in graded alcohol d embedded in Epon plastic A fine saw was d to divide each turn into several different locks These were mounted on Epon blanks for sectioning Free hand sections were made for light microscopic study, a Porter-Blum Ultratome was used for making thin sections Ultra thin sections were stained with uranyl acetate and lead citrate, and the study was done under a JEM-7 electron microscope

The serial transverse sections (20 μm) of the brain stem were made by cryostat, and Nauta staining was performed in order to investigate the nerve degeneration and also the depth and extent of the surgical lesion

## RESULTS

Severe nerve fiber degeneration within the organ of Corti was observed in the subjects two weeks after the transection of crossed OCB In the nerve endings to the outer hair cells, accumulation or reduction of vesicles, vacuole formation swelling of mitochondria, rupture of the membrane and appearance of osmiophilic substance, were observed to variable extents and degrees (Figs 1, 2, 2A, 2B, and 2C) These degeneration findings resemble those previously reported in the guinea pig and cat Three and one half weeks after the OCB transection, the complete disappearance of the nerve ending was observed in some areas Even though the nerve ending disappeared completely, the subsynaptic cisterna remained in the outer hair cells Degenerated cell debris (which was floating within the corti lymph space) was also found

Fiber reduction of the inner spiral bundle was found in the basal turn Thus, the existence of OCB in that particular region was confirmed (Fig 3) Likewise, few crossed OCB nerve endings were found at the inferior portion of the inner hair cells (Fig 4)

Degeneration of the tunnel spiral bundle was clearly observed (Fig 6) More degeneration was found in the lower turns than in the upper turns

With regard to the radial fibers within the Corti tunnel, interspecies difference exists In the guinea pig for example, both efferent and afferent nerve fibers exist together In the cat the tunnel radial fibers are considered to be basically efferent fibers, the afferent nerve fibers run inferiorly along the basal membrane being surrounded by pillar cells The present findings in the squirrel monkey resemble those in the cat The upper radial fibers belong to the efferent fibers (Fig 7) and a part of the afferent fibers run inferiorly, being surrounded by pillar cells (Fig 8)

The majority of the efferent nerve endings (which are attached to the inferior portion of the outer hair cells) in the lower basal turn belong to the crossed OCB Within the upper basal turn about a half of the nerve endings belong to the crossed OCB, however crossed OCB terminals are scarce at the middle turn and above

Within the upper basal to middle turns (espe-




Fig 1 Electronmicrograph exhibits the different forms of nerve endings on an outer hair cell (*H*) Two large nerve endings (*E*) which contain vesicles and mitochondria, and one small nerve ending (*A*) can be seen Squirrel monkey normal subject The black scale bar indicates 1 μm (in all figures)

cially 15-20 mm from the basal end) some efferent nerve endings attach to the supranuclear portion of the outer hair cells These particular nerve endings belong to the crossed OCB This finding is considered to be specific to the squirrel monkey (Fig 9)

In summarizing the above described findings, about 70% of the inner spiral bundle in the lower basal turn belong to the crossed OCB approximately the same percentage was found in the tunnel spiral bundle Some nerve endings of the crossed OCB exist around the inner hair cells In general, within the upper turns there is a reduction of the crossed OCB endings

Regarding the nerve terminals attached to the outer hair cells about 90% belong to the crossed OCB at the lower basal turns about 50% or more at the upper basal turns, however at the middle turn and above most of the nerve endings belong to homolateral OCB (Fig 10)

After studying the serial transverse sections of the brain stems of squirrel monkeys it was

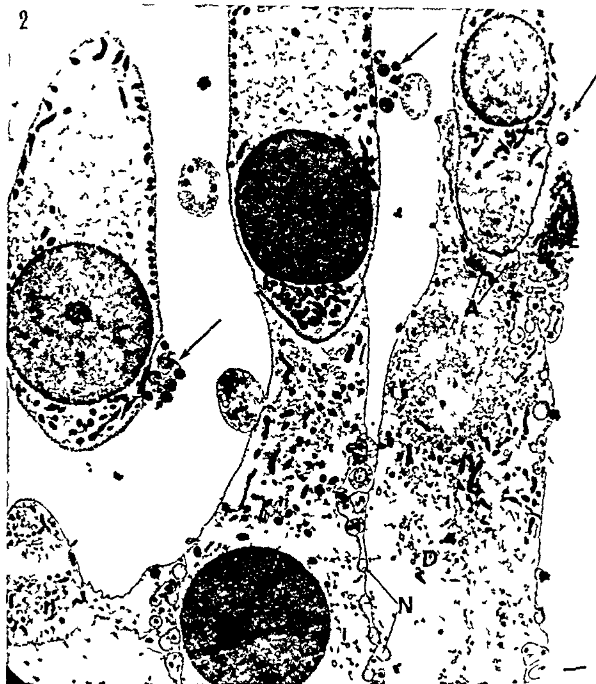



*Fig 2* This electronmicrograph demonstrates that three nerve endings (*arrows*) are in an advanced stage of degeneration 2 weeks after the transection of the crossed OCB This figure displays the area of outer hair cells from the upper basal turn (about 10 mm from the basal end) The one efferent (probably ipsilateral) nerve ending (*E*) and afferent nerve endings (*A*) at the right have an intact appearance *N* afferent nerve fibers *D* Deiters cell Squirrel monkey B9E




Fig 2A C Higher magnification views of parts of Fig. 2 showing the degenerate I trans-lateral efferent nerve endings on the outer hair cells


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found that the facial genu were relatively small in size and were usually located between 5 and 7 mm from the obex (Fig 11) It was found that all of the midline sections were extended well beyond the level of the edge of the facial genu (1-2 mm), both rostrally and caudally Therefore, if the crossed OCB goes across the midline at the region of the facial genu, it can be assumed that the majority of the crossed OCB were properly cut Out of 10 subjects, degenerated nerves (immediately beneath the facial genu) were seen in 3 and another 3 showed uncertain but possible degeneration

## DISCUSSION

Studies in other animal species have shown that the large nerve endings of the outer hair cells degenerate after cutting the OCB Kimura & Wersall (1962) found degeneration in some of the large nerve endings as well as some afferents to outer hair cells and some nerves in the tunnel bundle in the guinea pig after transection of crossed OCB Iurato (1962) reported that the large nerve endings disappeared within 8 days after the OCB section in the rat Smith & Rasmussen (1963 1965) by using the chinchilla, studied the *degeneration behavior of both translateral and* ipsilateral OCB between 2 and 35 days post transection They reported that the initial degeneration sign could be found within nerve fibers and large nerve endings 3 to 4 days post transection, and almost complete disappearance of cell debris 21 days post operation Spoendlin & Gacek (1963) also studied the efferent nerve system in the cat cochlea after cutting the homo and contralateral OCB Spoendlin (1969) further found that not only had a great number of the efferent endings at the outer hair cells degenerated but also the number of internal spiral fibers seemed to be reduced after the transection of the crossed OCB Inasmuch as inter-special difference exists, there is reason to utilize higher mammals such as subhuman primates for the study of OCB function

In the cat, approximately 2 weeks after the transection, the terminal degeneration was usually complete, with the exception of occasional small cell debris the nerve endings and fibers had completely disappeared (Spoendlin 1969 Igarashi et al, 1972) In the present study we have investigated the squirrel monkey cochlea 1-3½ weeks after the transection of the contralateral OCB The obtained results were fairly similar to those of the cat studies and the terminal degeneration was usually complete 2 weeks after the transection In the two cases 3½ weeks after the transection, nerve endings and fibers had almost completely disappeared therefore, it was considered that the survival time period in the present study was the appropriate time to investigate the distribution pattern of the OCB terminals in the squirrel monkey

Iurato (1962) described how the crossed OCB innervates only the outer hair cells in the rat According to Rasmussen's report (1960) approximately 4/5 of the fiber population of the OCB originate in the crossed bundle from the *contralateral accessory olivary nucleus, the re*maining 1/5 originate at the uncrossed bundle from the homolateral S shaped olivary segment in the cat

In the present investigation of the squirrel monkey, it was found that about 70 % of the inner spiral bundle belongs to the crossed OCB at the lower basal turns The crossed OCB partly innervates inner hair cells (directly) About 90 % of all efferent endings to the outer hair cells belong to the crossed OCB within the lower basal turns The innervation percentage of the crossed OCB relatively decreased within the upper turns, and the population from the homolateral OCB increased

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*Fig 3* Electronmicrograph demonstrates the lower portion of the inner hair cell The specimen was obtained about 6 mm from the basal end Inner spiral bundles below the inner hair cell (*IH*) have partly disappeared Two weeks post OCB transection Squirrel monkey D9A

*Fig 4* This electronmicrograph exhibits the lower portion of the inner hair cell (*IH*) The specimen was obtained about 10 mm from the basal end 2 weeks post OCB transection. Squirrel monkey A9B A nerve ending (*E*) has disintegrated *P* pillar cell

*Act*

5

6

P

P

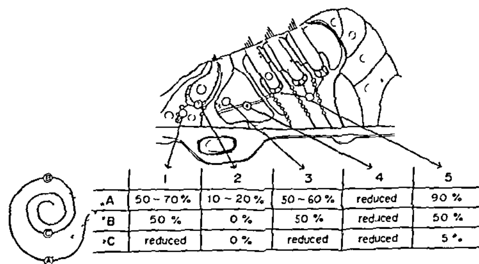
*Fg 5* Electronm crograph demonstrates tunnel sp ral bundle (*arrow*) *P* p llar cell Squ rrel monkey normal subject *Fg 6* The electronm crograph exh b ts the cond t on of the tunnel sp ral bundle 2 weeks after the OCB transect on Not ce degenerat ng efferent nerve f bers which belong to the crossed OCB (*a row*) The specimen was obta ned from an area about 8 mm from the ba al end *P* p llar cell Squ rrel monkey A9B




*Fig 7* This electronmicrograph demonstrates the degenerative changes in the part of the tunnel radial fibers (*arrow*). The specimen was obtained about 15 mm from the basal end some 3½ weeks post OCB transection. Squirrel monkey B3B

*Fig 8* Electronmicrograph shows the tunnel basilar fiber (*arrow*) which is entirely embedded in invagination of the pillar cells (*P*). Squirrel monkey B2B

*Fig 9* Electronmicrograph demonstrates a disintegrated nerve ending (*arrow*) in the supranuclear portion of an outer hair cell (*H*) The subsynaptic cisterna along the hair cell membrane appears to be normal Other efferent (*E*) and afferent (*A*) endings show no change About 20 mm from the basal end 2 weeks post OCB transection Squ r rel monkey B2E (A) Higher magnification view of the disintegrated nerve ending (*arrow*) in Fig 9



| | 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|---|
| A | 50 ~ 70 % | 10 ~ 20 % | 50 ~ 60 % | reduced | 90 % |
| B | 50 % | 0 % | 50 % | reduced | 50 % |
| C | reduced | 0 % | reduced | reduced | 5 % |

*Fig 10* Innervation from crossed olivo-cochlear bundle

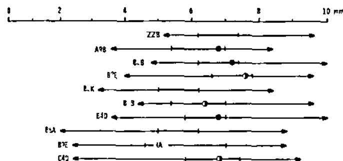



*Fig 11* This figure displays the rostro-caudal extent of midline lesions in squirrel monkeys The abscissa indicates the rostral distance from the obex Heavy line segments indicate the area of facial genu Nerve degeneration (by Nauta staining) was seen in cases with black circles while half black circles indicate possible degeneration (*A*) artefact

The degenerating condition or disappearing percentage of nerve fibers and endings within the organ of Corti varied among the different individuals One reason for this is probably the different extent of the midline sections at the brain stem or this is certainly due to the intra-individual variance

After the OCB transection in the cat, Spoendlin & Gacek (1963) and Spoendlin (1969) concluded that the efferent exist only in the upper tunnel radial bundle while the basilar bundle belongs to the afferent At the floor of the Corti tunnel, the basilar afferent fibers are usually embedded in invagination of the pillar cells, most of these take a short spiral course before they pass between the outer pillars In the guinea pig there are afferent fibers crossing the tunnel at a higher level according to Kimura & Wersäll (1962)

In the present study of the squirrel monkey, some of the basilar fibers are also embedded in invagination of the pillar cells just as in the cat, therefore, the basilar fibers might be afferent in nature On the other hand, some of the tunnel radial fibers degenerate after the midline transection, therefore, these radial fibers are considered to be efferent in nature Ishii et al (1967), described that both the upper tunnel radial bundle and the basilar radial bundle seemed to contain intense acetylcholinesterase activity in the squirrel monkey, therefore they suspected that both of these tunnel bundles contained efferent fibers in the squirrel monkey The discrepancy of this point remains to be determined

The nerve endings to the outer hair cells usually attach to the inferior portion of the cell body However, in the present study of the squirrel monkey, some of the efferent nerve endings attached to the supra-nuclear region of the outer hair cells at the upper basal and middle turns All of these endings belonged to the crossed OCB No such description was found in the rat, mouse, guinea pig, chinchilla or cat, therefore, it is considered that these supra nuclear nerve endings might be specific to the squirrel monkey

## ZUSAMMENFASSUNG

Bei einem Totenkopfaffen (*Saimiri sciureus*) wurde nach einem Querschnitt des Bundels am Boden der vierten Kammer die Verteilung der Enden des gekreuzten Olivo-Cochlea Bundels untersucht, und zwar elektronen mikroskopisch Ungefahr 70 % des inneren Spiralen-Bundels gehorten zu dem gekreuzten Olivo-Cochlea-Bundel der unteren Grundspiralen Ungefähr 90 % aller Enden, die nach aussen zu den ausseren Haarzellen fuhren, gehören zu dem gekreuzten Olivo-Cochlea-Bundel innerhalb der unteren Grundspiralen Der Prozentsatz der Nervenverteilung des gekreuzten Olivo-Cochlea Bundels ist dementsprechend geringer innerhalb der oberen Spiralen Nach Untersuchung von [illegible] Querschnitten des Gehirnstammes [illegible]

## REFERENCES

Gacek, R R, Nomura, Y [illegible] cholinesterase activity [illegible] acoustic nerve *Acta Otolaryng* [illegible]
Hilding, D & Wersäll [illegible] relation to the [illegible] *Otolaryng* (Stockh) [illegible]
Igarashi M, Al[illegible] 1972 Behavior [illegible] of crossed o[illegible]

tone threshold and perceptual signal to-noise ratio *Acta Otolaryng* (Stockh) *73*, 455

Ishii, T, Murakami, Y & Gacek, R R 1967 Histochemical study of the acetylcholinesterase activity in the inner ear of the squirrel monkey *Acta Otolaryng* (Stockh) *64*, 267

Iurato, S 1962 Efferent fibers to the sensory cells of Corti's organ *Exp cell Res 27*, 162.

Iurato, S, Smith, C A, Eldredge, D H & Henderson, D 1968 Electron microscopic observations and cochlear potentials after section of the crossed olivo-cochlear tract in the chinchilla *Fourth European Regional Conference on Electron Microscopy*, p 561

Kimura, R S & Wersall, J 1962 Termination of the olivo-cochlear bundle in relation to the outer hair cells of the organ of Corti in the guinea pig *Acta Otolaryng* (Stockh) *55*, 11

Rasmussen, G L 1953 Further observations of the efferent cochlear bundle *J Comp Neurol 99*, 61

Rasmussen, G L 1960 Efferent fibers of the cochlear nerve and cochlear nucleus In *Neural mechanisms of the auditory and vestibular systems* (ed G L Rasmussen & W F Windle), p 105 C C Thomas, Springfield, Ill

Schuknecht H F, Churchill, J A & Doran, R 1959 The localization of acetylcholinesterase in the cochlea *Arch Otolaryng* (Chic) *69*, 549

Smith, C A & [illegible]

*Anat Rec* [illegible]

— 1963 Recent observations on the olivo-cochlear bundle *Ann Otol 72*, 489

— 1965 Degeneration in the efferent nerve endings in the cochlea after axonal section *J Cell Biol 26*, 63

Spoendlin, H H 1967 The innervation of the organ of Corti *J Laryng 81*, 717

— 1969 Innervation patterns in the organ of Corti of the cat *Acta Otolaryng* (Stockh) *67*, 239

Spoendlin, H H & Gacek, R R 1963 Electronmicroscopic study of the efferent and afferent innervation of the organ of Corti in the cat *Ann Otol 72*, 660

Terayama, Y & Yamamoto, K 1971 Olivo-cochlear bundle in the guinea pig cochlea after central transection of the crossed bundle *Acta Otolaryng* (Stockh) *72*, 385

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# SOURCE OF THE $P_1$ COMPONENT OF THE COCHLEA ROUND WINDOW RECORDING

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*Abstract* The cochlear round window potentials of cats and guinea pigs have been studied in their response to a short click stimulus coupled with specifically placed neural lesions With the destruction of a specific area of [illegible] observation in contrast to the extensive recovery observed within 2 hours after lesions were made at the internal auditory meatus (including the vestibular nerve and OCB fibers) The lack of recovery in the animals in which lesions were placed in the cochlear nucleus may be explained on the basis that the responsible neurons in this area were destroyed Since the $P_1$ potential has a short latency with respect to the termination of microphonic activity and the changes are seen only with lesions placed peripherally to or at the specific cochlear nucleus area it is proposed that the $P_1$ potential possibly represents an activity of a peripheral coding or inhibitory mechanism

Davis 1957 presented an interpretation of the recordings obtained from the scalae of the cochlea and from the round window Many investigations of the cochlea have been based upon the use of these various electrical potentials recorded from the cochlea As the application of these methods is extended it has become evident that limitations exist concerning their interpretation During the past few years a number of experiments have been published dealing with the extension of the knowledge of what these recordings may represent (Dallos et al, 1971 Lawrence 1959, Salomon & Elberling 1971 Sohmer et al, 1971)

From this study evidence is offered towards extending the information concerning the usefulness of volume recording from the round window (r w ) in response to a short click stimulus It is recognized that volume recordings are an averaging of all the active components in the recording area under the electrode Consequently the recording has limitation when the components are to be interpreted Although this is the case with the potentials we are dealing with it is proposed that when a particular portion of the tracing is consistently affected by an experimental change it is possible to assign some specific correlate to it Our interest in these experiments is particularly concerned with the positive ($P_1$) component We have studied the influence of placing lesions along different parts of the efferent innervation at the level of the midbrain It is the aim of this study to learn whether the $P_1$ potential is associated with any of these sites

The interest of the ENT Department clinical personnel in the use of human intra aural cochlear potential recordings as a diagnostic aid has been fostered due to the development of signal averaging techniques (cochleograms) Therefore it was deemed important that what information we obtained concerning the possible interpretation of these potentials should be presented

This study was supported in part by a research grant from the National Institute for Neurological Disease and Blindness WB 06261

## METHODS

### *Animal lesion surgery*

The experimental animals, cats (20) and guinea pigs (9) were anesthetized with intraperitoneal

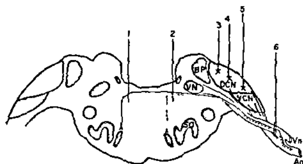



*Fig 1* Diagrammatic representation of the placement of various lesions in the cat auditory innervation Lesion *1* is placed to interrupt the contralateral olivocochlear fiber, lesion *2* placement will section both ipsilateral and contralateral olivocochlear bundle fibers, lesion *3* is in the medial pole of the cochlear nucleus as observed from a dorsal exposure, lesion *4* is placed in the cochlear nucleus mid portion of the dorsal exposure, lesion *5* is placed at the lateral pole of the observable field and lesion *6* is a section of the vestibular nerve at the internal meatus

injections of 0 7 ml/kg Dial Urethane solution (Ciba) They were placed into a Kopf stereotaxic head holder for orientation purposes A posterior fossa craniotomy was performed, by carefully removing occipital bone from the occipital crest to the foramen magnum and laterally out to the occipital condylus After splitting the dura, over the cerebellum, the cerebellum was removed by suction to expose the fourth ventricle and adjoining parts

This exposure allowed visualization of the ...al surface of the VIII nerve from its emer- ... rostro-ventral to the cochlear nucleus

Lesions were placed as shown in Fig 1 The sequence of lesions (refer to Fig 2) in each group of animals was performed as follows lesion 6 alone with a wire loop (5 cats), lesions 1, 2, 3, 4 and then 5 performed with a wire loop (6 cats, 5 guinea pigs), lesions 3, 4 and 5 performed with a bipolar (1 mm distance) electrode, current 2 milliamps, duration 15 sec (5 cats), lesions 3 4 and then 5 performed with the injection of a phenol solution (0 02 ml of 20% phenol in glycerin) with a 27 gauge hypodermic needle (4 cats) and the sectioning of the VIII nerve at the internal meatus with a wire loop (4 guinea pigs)

### *Recording procedure*

The round window potential measurement was obtained with a unipolar silver tipped spring loaded electrode placed at the base of the round window The approach was made through a lateral exposure of the bulla while the indifferent electrode was attached to the cranium The animal was grounded through the mouth clamp The cochlea round window potential was amplified with a Grass P9 capacity coupled preamplifier and recorded on a Tektronic Model 565 Oscilloscope Using the control tracing as a guide the time relationships for $N_1$ and $P_1$ peak voltage were determined (Fig 3, 1 2 and 1 6 msec respectively from the onset of the microphonics) These times were then used to locate the approx

The μV Value of Cochlea N and P₁ Potentials Following Lesions Placed In Various Parts of the Cochlea Innervation

| Methods of Lesions | | Control | 1 COCB | 2 COCB HOCB | 3 Coch Nuc Medial | 4 Coch Nuc Middle | 5 Coch Nuc Lateral | 6 Vest Nerve |
|---|---|---|---|---|---|---|---|---|
| Wire Section | N | 357 ± 82 | 156 ± 30 | 164 ± 43 | 164 ± 37 | 148 ± 40 | 220 ± 80 | 434 ± 106 |
| | P | 194 30 | 70 ± 23 | 78 ± 34 | 70 ± 30 | 68 ± 23 | 96 ± 20 | 11 ± 33 |
| Electric Coagulation | N | 190 30 | | | 235 ± 75 | 210 ± 50 | 285 ± 66 | |
| | P | 45 2 | | | 202 56 | 5 ± 37 | 120 ± 65 | |
| Phenol Injection | N | 276 ± 33 | | | 300 47 | 332 ± 59 | 362 ± 70 | |
| | P | 123 ± 17 | | | 120 12 | 129 ± 15 | 97 ± 10 | |
| Representative Tracing | | | | | | | | |

*Fig 2* These are the mean values ± standard error of the amplitude (μV) of the negative potential ($N_1$) and the positive potential ($P_1$) recorded from the cat cochlea round window Each group of animals served as their own controls The lesion placements are as indicated in Fig 1 The tracing inserts are examples of the potentials obtained from the cochlea under the condition indicated The $N_1$ amplitude and delay were not significantly changed by the lesions which produced $P_1$ alterations The abbreviations used are COCB (contralateral olivocochlear bundle), HOCB homolateral olivocochlear bundle) Coch nuc (cochlear nucleus) vest (vestibular) *a* (wire loop lesions experiment series) *b* (electrical coagulation experiment series)

imated point for the measurement of the magnitude of the negative potential ($N_1$) and positive potential ($P_1$) of subsequently measured distorted tracings Since the experimental conditions concerning the sound input for each experiment were kept constant the delay time with the tracings were also expected to be constant (Fex, 1965) The experiments were conducted in an electrically shielded Industrial Acoustic Company Chamber Model 5A Permanent records were filmed with a Grass C4-K Camera for later measurement Paired one tailed *t*-test was performed comparing each group with its own control values using $P<0\ 05$ as the acceptable level for indication of a significant difference

*Stimuli*

A click stimuli was generated to excite the r w potentials The click was generated in the TDH 39 earphone by a square wave (0 05 msec duration 0 05 volts) placed across the terminals, generated by a Grass S8 stimulator Fig 4 shows the spectral peaks of the click with the measuring microphone in place of the animals ear The click, when generated in the earphone placed next to the left ear, with the ear bar removed, resulted in a reproducible cochlear

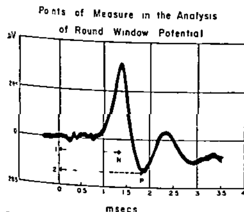



*Fig 3* This is a reproduction of a tracing obtained from the round window with the experimental system described in Methods The time relationship used for measurement of the appearance of the peak of the negative potential (N) and the positive potential ($P_1$) in control tracings is indicated. These measurements (1 2–1 6 msec latency after the MC) were then used to locate the point of measurement to obtain the extent of the distortion of the $P_1$ potential produced by the various lesions

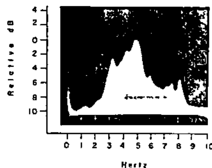



*Fig 4* The spectral analysis of the TDH 39 earphone output without an ear bar is shown The earphone was repetitively stimulated with 0 05 V 0 05 msec duration square wave pulse The spectrograph was obtained with a 1/2 inch Bruel & Kjaer Condenser Microphone connected to a Tektronic Spectrum Analyzer 3L5 model The peak output is slightly below 5 kHz with several lesser peaks through the spectrum

potential complex The intensity selected produced a tracing with a low amplitude microphonic tracing, and a prominent $N_1$ and $P_1$ potential (see Fig 3) The intensity of the sound stimuli was not sufficient to cause eliciation of the middle ear reflex and was calculated to be a peak value of $-26$ dB re 1 Volt = 0 0 dB The sound stimuli parameters and environment for each experiment were kept constant

*Histology*

The placement of the lesions in the cochlear nucleus were identified by the use of histological preparations The cat brain was perfused with saline at the end of the experiment and then fixed with neutral formaldehyde solution The fixed brain was trimmed to a block containing the cochlear nucleus and embedded in Paraplast by the usual Autotechnicon procedure Serial 10 $\mu$m sections were then stained with Hema toxylene and Eosin (see Manual of Histological and Special Staining Techniques, 1949) and mounted for light microscopy The guinea pig cochlear nucleus were not studied histologically

## RESULTS

Measurements of $N_1$ and $P_1$ magnitudes after different lesions are presented in Fig 2 Each

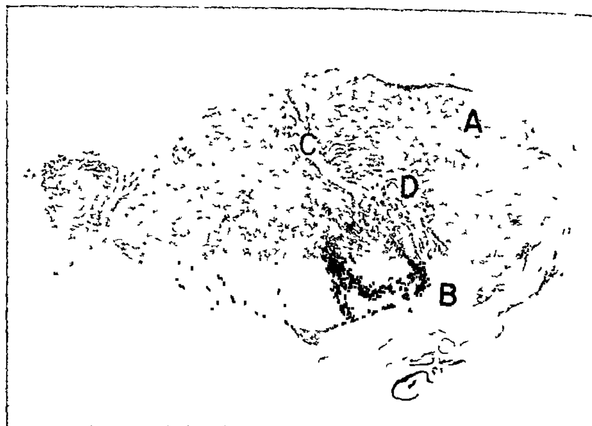



*Fig 5* A parasagittal section through the location of the lesion placed in the peripheral portion of the cochlear nucleus The lesion was produced with a 1 mm diameter electrode and a current of 2 mA for 15 sec The tissue is 10 μm thick stained with hematoxylene and eosin (A) dorsal cochlear nucleus (B) ventral cochlear nucleus (C) electrode pathway (D) lesion location Magnification is ×23

lesion as depicted in Fig 1, interrupted the efferent innervation at various levels of its course, from the segment of the olivary nucleus to the cochlea The lesions which produced a significant loss of the positive potential ($P<0.05$) were those placed either in the vestibular portion of the eighth nerve (lesion 6) in cats the VIII nerve complex in guinea pigs and in the lateral part of the dorsal aspect of the cochlear nucleus (lesion 5) in cat and guinea pigs Lesions which interrupted the more central pathways either of contralateral or ipsilateral efferent fibers produced no changes associated with the positive wave (lesions 1, 2 3 and 4)

Lesions placed in the lateral part of the dorsal cochlear nucleus (type 5) by either a wire section, phenol injection or electrical coagulation all produced the same initial loss of the $P_1$ potential (see Fig 2) These procedures do not affect the $N_1$ latency nor do they significantly change its amplitude ($P<0.05$)

The rate of $P_1$ recovery is different after various lesions When lesions were placed at the level of the vestibular nerve (lesion 6) extensive recovery of the $P_1$ potential occurred in a period of 2 hours (Daigneault et al 1968) Phenol injections into the lateral 1/3 of the cochlear nucleus as observed from a dorsal exposure (lesion 5) also showed a similar degree of reorganization of the $P_1$ wave In contrast with the electrical lesions placed in the same region the distorted potential remained the same during a 4 hour observation period

The histological evidence for localization of the area of the cochlear nucleus responsible for the changes is presented in Fig 5 The location is in the area where the dorsal cochlear nucleus and the caudal portion of the ventral nucleus

join peripherally. The cells at that location which would have been injured are small spherical cells and multipolar cells as described by Osen (1969). Afferent fibers of the descending branch from the basal turn are found in this area of the cochlear nucleus of the cat and the monkey (Moskowitz & Liu 1972).

Similar experiments performed upon guinea pigs produced parallel results. Only lesions associated with the lateral 1/3 of the cochlear nucleus, as viewed from the dorsal surface, and the VIII nerve caused the distortion of the cochlea tracings. In our hands, it was not possible in the guinea pig to obtain the changed pattern with only vestibular nerve section. The acoustic nerve had to be included before the extent of distortion equaled that observed in the cat.

## DISCUSSION

The proper utilization of round window tracings, for either research laboratories or as a human diagnostic procedure, will require accurate interpretations of the various potential components. This study extends our earlier work (Daigneault et al., 1968) which dealt with changes produced by vestibular nerve lesions upon the $P_1$ components of the r.w. tracings. From our experience with pharmacological experiments which have dealt with possible mediators or intoxicants of the cochlea we have found that the extent of possible interpretations of the tracings are limited. The present data indicate that the positive wave ($P_1$) is predictably affected by lesions placed peripheral to or at a specific part of the cochlear nucleus. The resulting changes in the electric pattern may be the result of changes produced in or near the cochlea. These may be either physical or neural changes induced by the lesion and reflected in the recording. Since some procedures produce either transient or prolonged (4 hours) changes in the r.w. pattern, the neural mechanism seems to be a plausible one.

As Rasmussen stressed by quoting Lorente de Nó (Rasmussen & Windle, 1965) "it is most urgent that we obtain knowledge of minute structure to enable proper interpretation of physiological data". The clue that the $P_1$ generating system can be associated with a part of the cochlear nucleus and possibly an efferent innervation helps to guide the direction the study should take. By some mechanism, it seems that a peripherally active system is responsible for an inhibition with a rapid onset of action (Mousheghian et al., 1971). The situation which may be operating at this peripheral site may be either that neurons are interacting upon other neurons in the spiral ganglia or with those in the cochlear nucleus, or the neurons may be interacting with an efferent fiber which courses through the area. Since the characteristics of the $P_1$ indicate that this is a neural phenomenon with a short delay time, it may well indicate that one of these mechanisms is operating. The short delay time of this phenomenon places it as a possible candidate for the mechanism postulated by other investigators (Katsuki et al., 1962, Mousheghian et al., 1971) that a peripheral inhibitory mechanism may well exist. A working example of this hypothesis would be that this is an active coding system present at this peripheral site accounting for signal processing as described by Lynn (Lynn & Sayers, 1970).

The characteristics of the $N_1$ portion of this tracing can be informative in the interpretation of these observations. The particular lesions which have been observed to produce changes in the cochlea potential ($P_1$) do not affect the latency of $N_1$ nor reduce the height of $N_1$. The lack of $N_1$ depression is evidence that the procedure did not initiate the usual olivocochlear bundle activity. It is possible that we are observing a limited action of the OCB or the activity of a different neuron system. It is proposed that the $P_1$ potential is either a separate neural system not previously recognized or a system interrelated in some way with a different level of activity of the efferent system. It is hypothesized that this neural system is responsible for the generation of the positive potential which may be active as a peripheral inhibitory or coding system.

A point of information from past experiments which has been a challenge to explain is the

extent of recovery of the $P_1$ potential as a consequence of sectioning the olivocochlear bundle in the vestibular nerve The persistent loss of $P_1$, produced by the electrocoagulation of specific areas of the cochlear nucleus, may provide a clue towards obtaining an answer From the experiments dealing with the selective section of the VIII nerve (Daigneault et al, 1970) it was evident that a minor portion of the fibers responsible for the $P_1$ potentials possibly exist in the acoustic nerve When our experiments were designed so that these fibers within the acoustic nerve were intact and thus available, for the still functional elements in spiral ganglia and the cochlear nucleus, a prominent degree of recovery was accomplished When a local anesthetic was used on the VIII nerve complex, recovery did not occur for the duration of the long-lasting local anesthetic (Dibucaine) (Daigneault et al, 1968) Therefore, when the elements responsible for the activity in the area of the cochlear nucleus are thus destroyed, or as with the local anesthetic, all the fibers are blocked for several hours, during that time the pattern of recovery does not occur This may be indicating that a role is being played by the neurons of the cochlear nucleus and/or the spiral ganglia and these are associated with the area to be considered as the generator of the positive potential It will be ecessary to further explore the anatomy of this rea in future experiments to verify if these assumptions are correct Such experiments utilizing light and electron microscopy are currently in progress in our laboratory with our attention directed to the spiral ganglia (Adamo & Daigneault, 1973)

With the development of procedures to obtain cochlea potential recordings from patients by using signal averaging techniques, the application of this technique in clinical work is being investigated The descriptive data of Aran (Aran et al, 1969) obtained from various patients with and without pathological conditions, have shown a wide variety of patterns The normal patterns are very much in agreement with the tracings obtained from the cat or guinea pig The abnormal patterns associated with pathological conditions are in most cases not interpretabl From personal communications with Dr Arar published reports of Salomon & Elberling (197 and similar recordings at our facility (Culle et al, 1972) some abnormal patterns similar t those reported in this investigation have bee observed from patients It is reasonable to expe that other examples of correlation between loca tion of pathology and the alteration of the shap of the cochlear potentials may be found in th future This type of information may make th human cochleogram a powerful diagnostic too

## ACKNOWLEDGMENT

The assistance of Dr C Berlin of the Gulf South Kreg Foundation in obtaining the spectral feature of th TDH39 earphone and the excellent laboratory workman ship of Mr Carlos Devia and Mr Clarence Foster Jr ar greatly appreciated

## ZUSAMMENFASSUNG

Katzen und Meerschweinchen wurden benutzt um der Einfluss spezifisch lokalisierter Nervenläsionen auf die durch ein kurzes Klickgerausch hervorgerufenen Aktions potentiale des Fenestra rotundum zu untersuchen Die Zerstorung eines spezifischen Gebietes des Nucleus cochlearis durch Elektrokoagulation fuhrte zu einem Ausfall der positiven Phase des Potentials Das Potential normalisierte sich wahrend einer Beobachtungszeit von vier Stunden nicht, im Gegensatz zu der weitgehenden

enthalt) angesetzt wurde Die mangelnde Reversibilität bei Tieren mit Lasionen im Nucleus cochlearis kann dam t erklart werden dass die verantwortlichen Neuronen dieses Gebiets zerstort worden sind Da das $P_1$ Potential eine kurze Latenz im Verhaltnis zur Ausdehnung der mikrophonischen Aktivität hat und da die Veränderungen nur auftreten wenn die Läsionen in der Peripherie vom oder im spezifischen cochlearen Kerngebiet angesetzt werden, kann gefolgert werden dass das $P_1$ Potential die Aktivität eines peripheren Coding oder Hemm Mechanismus darstellt

## REFERENCES

Adamo, N J & Daigneault, E. A 1973 Ultrastructural morphology of Schwann cell neuronal interrelationships in the spiral ganglia of cats *Amer J Anat 135* 73

Aran J M, Portmann C L, Delaunay J, Pelerin J & Lenior, J 1969 L'electrocochléogramme Methodes

et premiers resultats chez l enfant *Rev Laryng* (Bord) 90 615

Cullen, J K., Jr Ellis M S Berlin C L. & Lousteau R J 1972. Human acoustic nerve action potential recordings from the tympanic membrane without anesthesia *Acta Otolaryng* (Stockh ) *74* 15

Dagneault E. A Brown R D & Pruett J 1968 Cochlear round window recorded responses to acetyl choline and click stimulation following decentralization *Acta Otolaryng* (Stockh ) *66* 10

Dagneault, E. A Brown R D & Blanton J F 1970 Alteration of round window recorded $N_1$ by selective selection of the cochlear and vestibular nerves *Acta Otolaryng* (Stockh ) *70* 254

Dallos, P., Schoeny Z. G & Cheatham M A 1971 On the limitation of cochlear microphonic measurements *J Acoust Soc Amer 49* 1144

Davis H 1957 Biophysics and physiology of the inner ear *Phys ol Rev 37* 10

Fex J 1965 Auditory activity in uncrossed centrifugal cochlear fibers in cat *Acta Physiol Scand 64* 43

Katsuki Y Suga M & Kanno Y 1962 Neural mechanisms of the peripheral and central auditory system in monkeys *J Acoust Soc Amer 34* 1396

Lawrence M 1959 Stimulation deafness Cochlear patterns and significance of electrical recording methods *Otorhinolaryng 68* 1

Lynn P A & Sayers B McA 1970 Cochlear innervation signal processing and their relation to auditory time intensity effects *J Acoust Soc Amer 47* 575

*Manual of Histological and Special Staining Techniques* 1949 Armed Forces Institute of Pathology Second Edition The Blakiston Div McGraw Hill Book Co, Inc New York

Moushegian G Rupert A L Stillman R D & Weiss I P 1971 Inhibition in the auditory nerve? *J Acoust Soc Amer 50* 1558

Moskowitz, N & Liu J -C 1972 Central projections of the spiral ganglion of the squirrel monkey *J Comp Neurol 144* 335

Osen K K 1969 Cytoarchitecture of the cochlear nuclei in the cat *J Comp Neurol 136* 453

Rasmussen G L & Windle M F 1965 *Neural Mechanism of the Auditory and Vestibular System* Second Edition p 115 Ch C Thomas Springfield Ill

Salomon G & Elberling, C 1971 Cochlear nerve potentials recorded from the ear canal in man *Acta Otolaryng* (Stockh ) *71* 319

Sohmer A A Peake W T & Weiss T F 1971 Intracochlear potentials recorded with micropipets I Correlation with micropipet location *J Acoust Soc Amer 50* 572

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# ELECTRONYSTAGMOGRAPHY ON NORMAL PERSONS

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*Abstract* Twenty normal persons were studied by electronystagmography (ENG) for spontaneous positional and caloric nystagmus In addition a pendulum test was performed Spontaneous nystagmus was demonstrated in 3 persons Another 6 had positional nystagmus in 1-4 positions with a maximum angular velocity of 7 5°/sec The parameters (duration frequency and angular velocity) after caloric stimulation were analysed statistically The duration parameter showed least relative standard deviation There was no sign of a correlation between sex age, and the data measured No abnormal curves were found in the pendulum test On the basis of examples of centrally and peripherally conditioned nystagmus the pendulum test appears well suited for distinguishing between these types of nystagmus

By electronystagmography (ENG) it is possible to objectify spontaneous nystagmus (SN) or provoked nystagmus and to describe the parameters of the ENG curve In order to assess the s of such studies it is of decisive importance be in possession of comparable studies on ormals The present investigation was designed to present an electronystagmographic diagnostic programme and to assess the results of tests on normal persons

## MATERIAL AND METHOD

The material consists of 20 persons of an age distribution and sex ratio corresponding to the patients examined by ENG in the Department within a 5-year period (Fig 1) All the subjects were otologically normal had no complaints of dizziness or hearing impairment and were on no kind of medication

The recording was done as conventional electronystagmography, using a 4-channel writer Type ELEMA Mingograf 34 The mingograph was calibrated so that an ocular deviation of 10 corresponded to an excursion of 20 mm on the curve The subjects were tested after 30 minutes adaptation to the dark in order to obtain a stable corneal retinal potential (Munthe Fog 1963) They were investigated for SN and positional nystagmus (PN) and had caloric test as well as a pendulum test The examination was carried out in a dark room and the subjects had their eyes open and wore dark spectacles except in the pendulum test By this means we avoid inhibition of nystagmus due to ocular fixation and obtain at the same time better registration of nystagmus with open eyes To counteract voluntary inhibition of the nystaemus the subjects were asked to perform subtraction (1 000 − 7) during the test In the pendulum test the subject follows with his eyes a swinging pendulum in a dim red light

At the conclusion of the test it was checked whether the calibration had remained constant As stimulus in the caloric test we used irrigation with water at 44°, 250 ml for 30 sec The irrigation was done by an autom ly temperature and time regulated o Oto Type 06 C 643 K) As et al (1959) among better stimulus ing abnormalities system Indeed theoretically on by Lowenstein

By omitting

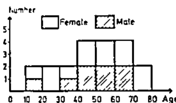



*Fig 1* Age-distribution and sex ratio of the material

diagnostic procedure is simplified, and the rare cases of pseudocaloric nystagmus are avoided (Greisen, 1972)

Since the various ENG parameters following caloric stimulation are attributed with different validity, and since their numerical calculation is different, we analysed statistically and compared the following parameters in our 20 normal subjects

I Maximum angular velocity of the slow phase which is induced by caloric action, whereas the rapid phase is a central compensation phase

II The number of nystagmic beats between 60 and 90 sec after starting the stimulus, as empirically this is the time interval in which the angular velocity of the slow phase as well as the frequency are at a maximum

III Total duration of caloric nystagmus meassured in seconds

Moreover, the difference between the right and left side in each person within the 3 parameters was calculated

The pendulum test was carried out by letting a recumbent subject follow with his gaze a swinging pendulum placed above the subject while simultaneously the ocular movements were recorded as usual in ENG The pendulum consists of a small steel ball suspended in a long, thin string, giving a swinging time of about 2 sec The distance of the pendulum from the person is about 40 cm Normally, the pendulum is made to swing horizontally and/or vertically in relation to the subject, with an excursion corresponding to 15–20° rotation of the eyes According to Maspetiol et al (1967) and Fonsman & Johansen (1971) normal persons and patients with peripherally conditioned nystagmus exhibit a regular sinus curve when watching the swinging pendulum, whereas a patient with centrally conditioned nystagmus shows an irregular sinus curve Thus, by this simple test it ought to be possible to distinguish between peripherally and centrally conditioned nystagmus

## RESULTS

Of the 20 normal persons, 3 had SN with 3 5°/sec of the slow phase as the maximum value The recorded SN was largely unchanged on examination in various positions The 3 subjects having SN were of the age groups 20–29, 50–59, and 60–69 years Another 6 subjects had PN in from 1 to 4 positions The maximum angular velocity of the slow phase was 7 5°/sec These cases were distributed on all age groups

The recorded data following caloric stimulation for the maximum angular velocity of the slow phase—number of nystagmic beats between 60 and 90 sec as well as the duration measured in seconds—were statistically analysed (with assistance from the Department of Data Processing) by Wilcoxon's signed rank test, acting on the hypothesis that the distribution is symmetrical around the mean value, and by the Kolmogorov Smirnov test for comparison with the normal distribution having the same mean value and standard deviation By these tests it was not possible to demonstrate a deviation from the normal distribution It was only concerning certain data for the maximum angular velocity of the slow phase that some doubt arose as to whether the distribution was normal It must be mentioned, however, that the size of the data does not afford the best possibilities for disclosing deviations from a normal distribution by statistical analysis If a transformation of the maximum angular velocity data was performed by calculating 10 × the logarithm of these values (Hinchcliffe, 1967), these logarithmic data were of a normal distribution with a smaller Kolmogorov-Smirnov fractile than the original data This is interpreted as an improved adaptation to a normal distribution When analysed in this way, the present material gave values practically

Table I *Statistical results of the single side values (caloric test)*

| Single side values Caloric (44°) | | Wilcoxon fractile | Kolmogorov Smirnov fractile | Common mean | Common S D | Relative deviation | Fractile 2 5 % | 97 5 % |
|---|---|---|---|---|---|---|---|---|
| Speed of slow phase | R | 0 521 | 0 941 | 23 9 | 16 29 | | −8 7 | 56 5 |
| | L | 0 277 | 0 343 | °/sec | °/sec | 68 2 % | °/sec | °/sec |
| *10 × log speed of slow phase* | R | *0 234* | *0 332* | *13 01* | *2 58* | *19 85 %* | 6 1 | 65 6 |
| | L | 0 629 | 0 336 | | | | °/sec | °/sec |
| Number of beats (60–90 sec) | R | 0 774 | 0 830 | 76 1 | 23 75 | 31 25 % | 28 | 123 |
| | L | 0 103 | 0 136 | | | | beats | beats |
| Duration | R | 0 014 | 0 173 | 176 23 | 31 87 | 17 9 % | 112 | 240 |
| | L | 0 074 | 0 553 | sec | sec | | sec | sec |

coincident with those of the Hinchcliffe material Furthermore, it was not possible to demonstrate *statistically a significant difference between right* and left values, and no correlation between sex, age, and the measured data could be found

From a statistical point of view it is expedient to select the parameter which has least relative standard deviation (the narrowest Gaussian curve) to express the normal range The results are apparent from Table I It is apparent that the duration shows the least relative standard deviation of 17 9 % If the duration in a normal material is plotted on probability paper, giving the mean value as well as the 2 5 % and 97 5 % [illegible] we get a straight line, from which it is [illegible]ly possible to estimate with how much [illegible]obability a measured value on one side is nor[illegible]al As may be seen from Table I, the 2 5 % and 97 5 % fractiles for duration were 112 sec and 240 sec respectively and the mean value 176 sec (Fig 2) Thus, values below 112 sec indicate hypofunction, whereas values above 240 sec indicate hyperfunction (SN and/or directional preponderance (DP))

*The measured data for the three parameters* show a marked difference between the values from one person and those of another within the individual parameter—and also in some cases a marked difference between the values for the *right and left side in the individual subject* Since theoretically it would be expected that in normal persons the difference between the values for the right and left side in each individual is 0 or close to 0, it seems natural to express the difference between the right and *left* side in per cent of the two sides' numerical sum value on the background of the above mentioned marked individual difference Something like this was suggested by Jongkees et al (1962) among others Since, moreover, it is *desired that the* difference values expressed in per cent should—if possible—be of a normal distribution the differences have to be calculated with opposite signs in order to obtain a symmetrical distribu

Table II *Statistical results of the difference values calculated in per cent (caloric test)*

| Difference values in % Caloric (44°) | Wilcoxon fractile | Kolmogorov Smirnov fractile | Mean | S D | Error of mean | Fractile 2 5 % | 97 5 % |
|---|---|---|---|---|---|---|---|
| Diff speed of slow phase | 0 462 | 0 125 | 7 % | 19 % | ±1 1 % | 45 % | +31 % |
| Diff number of beats | 0 474 | 0 243 | 3 % | 11 % | +6 3 % | 25 % | +19 % |
| Diff duration | 0 729 | 0 566 | 6 % | 5 % | +4 6 % | 22 % | +10 % |

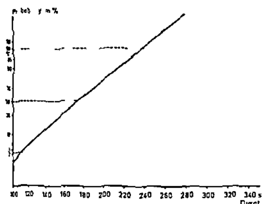
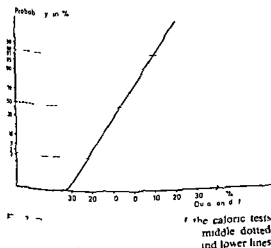



*Fig 2* Duration (single side values) of the caloric tests (44°) plotted on probability paper The middle dotted line represents the mean value—the upper and lower lines represent two times the standard deviation

tion around the mean Arbitrarily, we decided to call the right side values positive and the left side values negative This calculation showed that the differences fitted well in with a normal distribution The results are presented in Table II Here too, it is evident that the duration parameter for the difference values gives least standard deviation (8 %) In a way similar to that used for the values for each side, the difference values may be plotted on probability paper as a straight line with the 2 5 % and 97 5 % fractiles (Fig 3) Thereafter, the normality of calculated differences may be immediately estimated by plotting on the curve




F ... the caloric tests ... middle dotted ... and lower lines

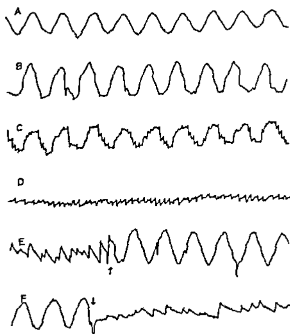



*Fig 4* Pendulum test (A) Regular sinus curve (B) Slightly irregular sinus curve (C) Serrated abnormal pendulum curve from a patient with epilepsy (D) SN from the same patient as C (E) and (F) show caloric induced nystagmus disappearing when the eyes follow the pendulum and re-appearing when the eyes are closed

Of the 20 normal persons 17 had a regular sinus curve in the pendulum test, as shown in Fig 4A Maspetiol et al (1967) distinguish between a normal, regular sinus curve and 6 different types of abnormal sinus curves In the present paper a distinction is made between a normal, regular sinus curve, a slightly irregular sinus curve, and an abnormal sinus curve Three out of the 20 normal persons had a slightly irregular sinus curve of the type shown in Fig 4B Two of these 3 subjects had SN and the third had PN

## DISCUSSION

The reported findings of SN and PN in ENG of normals have differed For instance, Stahle (1958) in a series of 50 normals, found none to have either SN or PN, and Jongkees et al (1962) detected SN in only one of 85 normal subjects (angular velocity not stated) According to several authors only a SN over a certain angular

velocity can be considered abnormal Bos et al (1963) stated that a SN exceeding 6°/sec angular velocity was highly suggestive of an abnormal condition, and Hinchcliffe (1967) found, among 20 normal persons, 2 who had SN, in both of them below 6°/sec angular velocity In the present material 3 persons had SN, though not exceeding 3 5°/sec angular velocity Another 6 had PN, maximum angular velocity 7 5°/sec

To assess an abnormal caloric reaction it is important to know the values for each side as well as the difference value, as these values supplement each other For instance, it is not always possible to conclude from an abnormal difference value which side, or whether both sides give an abnormal caloric reaction As is evident from Table II, all the mean values for the various difference parameters were negative, although they would be expected to be 0 or close to 0 Against this background an investigation was made for faults in the apparatus or procedure, but no such faults were found The phenomenon may be due to the relatively small material (cf the statistical error of the mean, Table II) or the fact that the right ear is always irrigated first This possibly entails a psychic inhibition due to fear of the unknown, a factor which is perhaps not present at the subsequent irrigation of the left ear

Since it was to be expected, inter alia on the basis of Lowenstein & Sand's study (1940), that in normal individuals there is a vestibular resting tonus, the vestibular apparatus of the two sides keeping each other in equilibrium, it is surprising to observe how marked a difference there may be between the caloric values for the two sides in the same normal person In view of the fact that the externally administered caloric stimulus is technically and physically identical on both sides, the marked difference between the two sides is probably due to the caloric energy administered to the semicircular canals being different nevertheless As shown by the values, the caloric test must be considered a relatively rough test

On the basis of an analysis of a normal material, the duration parameter seems most expedient From this it is not permissible to conclude direct that this will also apply to an abnormal material It is only by applying the parameters to such a material that it is possible to decide which is most expedient The duration—estimated on the basis of the ENG curves—posed no problem

The pendulum test does not represent a reflex, as does the caloric nystagmus, or a partial reflex, as does optokinetic nystagmus, but is a purely voluntary act, presupposing that the patient can see and follow the pendulum with his eyes This test showed that normals have either an entirely regular sinus curve or a slightly irregular one Those having a slightly irregular sinus curve had either a faint SN or a PN

Fig 4C presents an abnormal-serrated-asymmetrical sinus curve from a patient showing signs of epilepsy Fig 4D shows the registration of SN from the same patient as the one in Fig 4C It is obvious that the SN manifests itself on the sinus curve

Fig 4E represents the same normal subject as Fig 4A The first part of the curve shows a caloric reaction after irrigation of the right ear with 44° water for 30 sec At [↑] the eyes were opened, whereupon the eyes followed the movements of the pendulum, subsequently giving a regular sinus curve without any manifestation of the marked caloric nystagmus

Fig 4F is the continuation of E—after a regular sinus curve of several seconds' duration At [↓] the subject again closed his eyes, and thereafter the normal nystagmus was again manifest on the ENG curve

In deciding whether a patient has a vestibular or a central disorder, many authors base their deductions on the caloric reaction, a weakered caloric reaction often being interpreted as a sign of peripheral disease Studying midline lesions in the brain stem of cats Stroud et al (1971) demonstrated that such lesions may temporarily as well as permanently abolish the caloric reaction This, then, can hardly be a consistent indication of a peripheral disorder

The result of the pendulum test on normals, which revealed no abnormal curves, as well as

the examples shown in Fig 3 indicate that the pendulum test is an important aid in deciding whether nystagmus is of central or peripheral origin

## ZUSAMMENFASSUNG

Zwanzig normale Menschen wurden mit Elektronystagmographie auf Spontan , Lage- und kalorischen Nystagmus hin untersucht Daneben wurde ein Pendeltest ausgeführt Spontannystagmus wurde bei 3 Personen festgestellt Weitere 6 Personen hatten Lagenystagmus in 1–4 Positionen, wobei die Winkelgeschwindigkeit maximal 7 5 /sec betrug Die Parameter (Dauer, Frequenz und Winkelgeschwindigkeit nach der kalorischen Stimulation) wurden statistisch analysiert Der Dauerparameter zeigte die geringste relative Standarddeviation Es bestand kein Zusammenhang zwischen Geschlecht Alter und den gemessenen Daten Im Pendeltest wurden keine abnormen Kurven gefunden Aufgrund von Beispielen an zentralen und labyrinthar vestibularen Nystagmen wird angenommen, dass der Pendeltest zur Trennung von diesen Typen von Nystagmus geeignet ist

## REFERENCES

Bos J H, Oosterveld, W J, Philipszoon, A J, Vozza, J & Zelig S 1963 *Pract Otorhinolaryng* (Basel) *25*, 282.

Fonsman J & Johansen H 1971 Pendulnystagmografi *Dansk Oto laryngologisk Selskabs forhandlinger*

Greisen O 1972 Pseudocaloric nystagmus *Acta Otolaryng* (Stockh ) *73*, 341

Hinchcliffe, R 1967 Normal values for caloric tests using electro-oculography *J Laryng 81*, 2, 221

Jongkees, L. B W, Maas J P M & Philipszoon, A J 1962 Clinical nystagmography *Pract Otorhinolaryng* (Basel) *24*, 65

Koch, Hj, Henriksson, N G, Lundgren A & Andren G 1959 Directional preponderance and spontaneous nystagmus in eye speed recording *Acta Otolaryng* (Stockh ) *50*, 517

Lowenstein O & Sand A 1940 The mechanism of the semicircular canal A study of the responses of single-fibre preparations to angular accelerations and to rotation at constant speed *Proc Roy Soc Biol 129*, 256

Maspetiol, R, Semette, D & Jackowska Hedemann, A 1967 Epreuve du pendule et nystagmographie *Rev Laryng 88*, 43

Munthe Fog C V 1963 The dependence of corneo-retinal potential upon illumination *Acta Otolaryng* (Stockh ) Suppl *188*

Stahle, J 1958 Electro-nystagmography in the caloric and rotatory tests *Acta Otolaryng* (Stockh ) Suppl *137*

Stroud Malcolm H, Marovitz, William F & Leyton Orlando C 1971 Vestibular dysfunctions after mid line lesions in the brain stem of the cat *Ann Otol 80* 750

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# THE EFFECT OF VISUAL INPUT ON POST-ROTATORY NYSTAGMUS IN NORMAL CHILDREN

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*Abstract* The duration, total number of beats, and frequency of post rotatory nystagmus were measured in normal young children under different conditions of visual input After rotation in darkness, the effects of fixation without light, fixation in light, and light without fixation on the nystagmus evoked by an abrupt braking deceleration were studied The post rotatory nystagmus duration and the total number of beats were significantly reduced both by fixation without light and by light without fixation The findings were discussed in relation to mechanisms by which vestibular nystagmus may be suppressed

The suppressive effect of visual input on vestibularly-induced nystagmus has been well documented for adult subjects (Wendt, 1951, Collins, 1966, Marshall & Brown, 1967, Collins, 968, Hood, 1970, Pfaltz & Piffko, 1970) studies have not been reported for In the adult studies, visual *fixation* has been assumed to be the effective mechanism by which vestibular nystagmus is suppressed in *the presence* of visual input, however, the relative influence of *fixation per se* and the effect of *light* on the vestibulo-ocular reflex has not received comment Interest in the present investigation in normal children was generated by an earlier finding that the suppression of post-rotational nystagmus in autistic children (Pollack & Krieger, 1958, Colbert et al, 1959) was dependent on a free visual field and did not occur in darkness (Ritvo et al, 1969) In preparation for further studies of the interaction of visual and vestibular stimuli in autistic and other developmentally deviant children, this investigation explores the relative contributions of visual fixation and light to suppression of post rotatory nystagmus in normal children

## METHODS

### *Subjects*

Twenty-five 38–90 month-old (mean 56.4 months) normal children were studied for 3 sessions each The sessions occurred at 2 to 4 day intervals Sixteen were boys and 9 were girls

### *Stimulation*

In order to obtain data that would be comparable to that obtained in the studies of autistic children (Pollack & Krieger, 1958, Colbert et al, 1959, Ritvo et al, 1969), the conventional Barany procedure of *10 complete revolutions* in 20 sec followed by an abrupt stop was employed The subjects sat in an electromechanically driven and remotely controlled chair which was rapidly accelerated from rest to 180°/sec in complete darkness for 20 sec The horizontal semicircular canals were maintained in the horizontal plane by partial fixation of the subject's head with a padded occipital brace and an elastic head strap Subjects received 6 clockwise and 6 counterclockwise rotations during each

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Table I *The experimental conditions used to study the influence of visual input on post-rotatory nystagmus*

| Condition | | Purpose |
|---|---|---|
| 1 | Standard visual field with standard illumination | To determine the degree of suppression of nystagmus in light when fixation is possible but is not encouraged |
| 2 | Standard visual field with fixation object added | To determine if nystagmus suppression would be enhanced when deliberate fixation is encouraged |
| 3 | Pinpoint of red light with minimal light value in complete darkness | To study the effect of fixation in the absence of light |
| 4 | Goggles admitting light without visual pattern Fixation precluded Light intensity equivalent to Condition 1 | To study the effect of light without fixation |
| 5 | Same as Condition 4, but with lower light intensity | To study the effect of light intensity |
| 6 | Complete darkness | For comparison with other conditions This is the 'neutral' condition |

session At least 4 minutes elapsed from the end of one rotation to the beginning of the next Each pair of clockwise and counterclockwise rotations in absolute darkness was immediately followed by one of the visual conditions outlined in Table I In Condition 1, the chair always stopped facing an evenly illuminated white background The children received no special instruction other than to keep their eyes open and avoid head movements In Condition 2, a black cross was added to the white background directly in the line of vision when the chair stopped The children were encouraged to stare at the cross In Condition 3, they were encouraged to look at a pinpoint of red light directly in the line of vision when the chair stopped, the room remained absolutely dark and the point of red light provided no illumination In Condition 4, the subject wore frosted goggles while rotating in the chair When the lights came on, a bright white patternless field was visible but fixation was precluded Condition 5 was similar except that the illumination was less the subjects saw a dull gray patternless field In Condition 6, the room remained absolutely dark after the chair stopped *In Conditions 1 through 5, the* various types of light came on at the exact moment that the chair abruptly stopped Condition 1 was always the first and Condition 6 was a[illegible] last condition during each of the 3 sessions The sequence of Conditions 2–5 were varied during the 3 sessions to permit assessment of the effects of order of presentation

An adult was in the room with the child at all times but was out of the child's line of sight when the chair stopped The adult could "see" any head movement in the dark during rotation by *observing the relative position of two pin* points of red light (not visible to the child) attached to the side of the child's head

*Recording*

Horizontal eye movements were recorded bipolarly from silver-silver chloride electrodes at the outer canthi on a Grass polygraph using a paper speed of 10 mm/sec The d c coupling was modified so that a 3 sec time constant was induced Vertical eye movements and blinks were recorded on a second channel from leads placed directly above and below one eye

*Data sampling*

It was necessary to discard a certain trials because of (1) lack of [illegible] child, (2) drowsiness or falling as[illegible] movement during or after to keep eyes open, and (5) fa[illegible]

Most of the children cooperated very well but occasional trials were discarded because, e g, the child brought his hand up to his face, talked during the trial, etc Trials during which drowsiness or sleep occurred were discarded In addition to visual observation of the child when lights were on, drowsiness was determined by observing an absence of blinking combined with slow rolling eye movements on the electro oculogram (EOG) The absence of blink artifact was also used to determine that the child was not keeping his eyes open Trials were discarded whenever head movements were observed

Those trials in which Conditions 2 through 5 obtained (see Table I) were subjected to additional scrutiny Three of the investigators independently examined the EOGs for these conditions without knowledge of the age or identity of the subject to determine if ocular fixation did or did not occur A trial was discarded if it was determined that ocular fixation had occurred under Conditions 4 or 5 or had *not* occurred under Conditions 2 or 3 If the three investigators did not unanimously agree to discard or retain a trial, a final decision was reached after discussion If consensus could not be reached, the trial was also discarded The presence or absence of fixation was determined by evaluating the relative amplitude of the nystagmus [illegible] equate fixation during Conditions 2 and 3 [illegible] always associated with nystagmus amplitude [illegible] *ich was clearly smaller than that of Condi*tion 6 The presence of undesirable fixation during Conditions 4 and 5 was associated with a nystagmus amplitude which was similar to that of Conditions 2 and 3

Twenty-five of the scheduled 900 trials (25 subjects × 3 sessions × 6 conditions × 2 directions) could not be carried out due to lack of subject cooperation (21 of these involved Conditions 4 and 5, the children refusing to wear the goggles) Forty-five extra trials were carried out in addition to the 900 scheduled trials, making a total of 920 trials actually run Of these 920 trials, 137 were ultimately discarded for one or more of the reasons described above, leaving 783 successful trials for data analysis

Table II *The relative success in carrying out the different experimental conditions in children*

| | Number of trials attempted | Number of successful trials | Percent of successful trials |
|---|---|---|---|
| Condition 1 | 154 | 148 | 96 1 |
| Condition 2 | 153 | 133 | 86 9 |
| Condition 3 | 167 | 136 | 81 4 |
| Condition 4 | 152 | 112 | 73 7 |
| Condition 5 | 140 | 109 | 77 9 |
| Condition 6 | 154 | 145 | 94 2 |
| Total trials all conditions | 920 | 783 | 85 2 |

The majority of the 137 unsuccessful trials (88 trials) were discarded because the criteria for presence or absence of ocular fixation were not met Table II gives a measure of the degree of success in carrying out the different experimental conditions in young children

*Data analysis*

Both the duration of nystagmus and the number of nystagmic beats following the abrupt stop of the chair were measured

Two measures of duration were used These measures were developed during a preliminary phase of this investigation to take into account the variability of childrens' nystagmograms. The "uninterrupted" duration score is the duration in seconds from the end of rotation to the last nystagmic beat of a train of beats in which the time between the end of one beat and the beginning of the next does not exceed 1 0 sec [1] The "total" duration score is the duration in seconds from the end of rotation to the end of the last nystagmus beat of a series of 3 consecutive beats regardless of earlier disruptions in the nystagmogram

Two measures of the number of beats were

[1] A nystagmic beat is defined as a slow phase immediately followed by a fast phase Two qualifications of the [illegible] were made [illegible]

nystagmic beats longer than 1 0 sec was accepted [illegible] train of beats continued after the disruption for a period of time greater than the sum of the time of the disruption and the previous period of nystagmus

Table III *Group means and standard deviations of nystagmus durations for 22 normal children under 6 conditions of visual input*

| | Uninterrupted duration (sec) | | | | Total duration (sec) | | | |
|---|---|---|---|---|---|---|---|---|
| | Both directions | | Maximum direction | | Both directions | | Maximum direction | |
| Condition | $\bar{x}$ | ±S D | $\bar{x}$ | ±S D | $\bar{x}$ | ±S D | $\bar{x}$ | ±S D |
| 1 | 3 83 | 2.07 | 4 98 | 2 38 | 4 57 | 2 72 | 6 08 | 3 32 |
| 2 | 4 66 | 2 04 | 5 84 | 2 68 | 5 35 | 2 42 | 6 63 | 3 03 |
| 3 | 9 50 | 3 20 | 10 85 | 3 79 | 10 93 | 3 54 | 12.62 | 4 11 |
| 4 | 13 21 | 4 22 | 15 17 | 5 06 | 16 95 | 6 06 | 20 01 | 7 42 |
| 5 | 12 04 | 4 28 | 14 79 | 5 27 | 16 50 | 5 11 | 19 57 | 6 31 |
| 6 | 22 15 | 5 20 | 25 61 | 5 72 | 26 04 | 6 10 | 29 46 | 6 76 |

used (1) the total number of beats following the end of rotation, and (2) the frequency of beats (the number of beats per each 3 sec interval following the end of rotation) For the latter measure, if artifact obscured part of a 3 sec interval, that interval was treated as missing data in subsequent analyses

For each condition, two methods were used to summarize the data obtained from a maximum of 6 trials (one pair of CW and CCW trials per session for 3 sessions) The first was to take the mean of *all* successful trials Because of the possibility of directional preponderance, a second method was to calculate the mean of the *maximum* values from the pairs of CW and CCW trials from each session Since both methods yielded similar results, only the results using the first method will be reported in detail

## RESULTS

### *Directional preponderance*

To determine whether or not directional preponderance was present, $\chi^2$ goodness of fit tests were applied to all usable pairs of clockwise (CW) and counterclockwise (CCW) trials for each of the 25 children using the uninterrupted duration scores [1] The number of pairs of trials in which CW > CCW was compared with those in which CCW > CW for each child $\chi^2$ with 1 degree of freedom was greater than 3 84 ($P<0\ 05$) for only 4 of the 25 children To obtain a simultaneous test for the group of subjects, $\chi^2$ and degrees of freedom were summed for the 25 subjects giving $\chi^2=40\ 14$ which was significant ($P=0\ 05$) However, over 50 % of the $\chi^2$ for the group of children was accounted for by only 4 subjects

### *Habituation and the order of presentation of trials*

The possibility of habituation across the 3 experimental sessions was tested by comparing the mean nystagmus durations from each session for each of the 6 conditions between the pairs of sessions 1 and 2, 1 and 3, and 2 and 3 (*t*-test on paired dependent observations two-tailed) For both the uninterrupted and the total duration scores, there were no consistent changes across the 3 sessions In fact, only one *t* test was significant which is expected due to the number of tests performed

Similarly, computation of both the total number of beats and the frequency of beats revealed no clear pattern of habituation More paired *t* tests were significant, but most of these differences lost significance when a multiple range test was used to take into account the comparison between three days

Since the order of Conditions 2 u was varied on the three days, the

[1] Data were available for both the CW and CCW directions for 8 to 18 pairs of trials (mean 14 0 pairs) out of 18 possible pairs of trials

Table IV *Group means and standard deviations of number of nystagmus beats for 22 normal children under 6 conditions of visual input*

| | Total number of beats | | | | Frequency of beats (beats/sec) | | | |
|---|---|---|---|---|---|---|---|---|
| | Both directions | | Maximum direction | | Both directions | | Maximum direction | |
| Condition | $\bar{x}$ | ±S D | $\bar{x}$ | ±S D | $\bar{x}$ | ±S D | $\bar{x}$ | ±S D |
| 1 | 7 2 | 4 0 | 13 8 | 7 6 | 1 2 | 0 3 | 1 7 | 0 5 |
| 2 | 12 1 | 5 5 | 19 6 | 8 6 | 1 8 | 0 3 | 2 4 | 0 4 |
| 3 | 25 9 | 8 0 | 39 4 | 12 4 | 2 1 | 0 4 | 2 6 | 0 5 |
| 4 | 26 6 | 10 9 | 36 0 | 13 4 | 1 5 | 0 3 | 1 9 | 0 3 |
| 5 | 25 1 | 9 9 | 35 0 | 13 0 | 1 4 | 0 4 | 1 7 | 0 5 |
| 6 | 39 0 | 13 5 | 48 8 | 16 0 | 1 4 | 0 4 | 1 8 | 0 5 |

consistent effects of the order in which conditions were presented within days

*Age*

For the group of 25 children, correlation coefficients were computed between age and the mean nystagmus durations for the uninterrupted and total duration scores for each of the 6 experimental conditions There were no significant correlations between subject age and nystagmus response under any of the experimental conditions Similar results obtained for the total number and frequency of beats except that there was a very slight tendency for the total number beats to increase with age in Condition 6 mplete darkness) ($r = + 40$, $P \doteq 0 05$, two iled)

*Sex*

The mean uninterrupted nystagmus durations, the total number of beats, and the frequency of beats from all successful trials of the 16 boys were compared with those of the 9 girls (two sample $t$ tests with separate variances) for each experimental condition on each of the three experimental days There were no significant sex differences which were consistent across the 3 experimental days

*The effect of the visual input condition*

Since there was no habituation across the 3 experimental days and since order of presentation of conditions and sex and age of subject did not influence the nystagmus scores, all successful trials were average for each subject in each condition Twenty two of the 25 children had successful trials in all 6 experimental conditions

*Nystagmus duration* A two-way analysis of variance (ANOVA) was carried out across these 22 subjects and 6 conditions The ANOVA demonstrated that the conditions differed significantly ($P<0 001$) Table III shows the nystagmus durations for the different experimental conditions The Newman-Keuls Multiple Range Test was used to test the significance of differences between the different conditions The uninterrupted post rotatory nystagmus durations were significantly longer in complete darkness (Condition 6) than in all other conditions ($P<0 01$). Under both conditions of light without fixation (Conditions 4 and 5) and fixation without light (Condition 3), the nystagmus durations were significantly longer than in the two standard visual field conditions (Conditions 1 and 2) ($P<0 01$) During fixation without light (Condition 3), the nystagmus durations were significantly shorter than during both light without fixation conditions (Conditions 4 and 5) ($P<0 01$) The results were the same for the total durations

*Number of nystagmus beats*

Two-way ANOVAs also demonstrated that both the total number and the frequency of beats differed significantly under the 6 experimental

conditions Table IV shows the total number and frequency of beats for the different conditions Application of the Newman-Keuls Multiple Range Test showed that the results were essentially the same for the *total number of beats* as for the *duration scores* There were significantly more beats in complete darkness (Condition 6) than in all other conditions ($P<0\ 01$) and there were significantly more beats in Conditions 3, 4, and 5 than in Conditions 1 and 2 ($P<0\ 01$) Unlike the duration scores, there was no significant *difference in number* of beats when Condition 3 was compared with Conditions 4 and 5

The results of the analysis of the *frequency of beats* were substantially different Those conditions which encouraged ocular fixation (Conditions 2 and 3) were associated with a greater nystagmus frequency than all other conditions ($P<0\ 01$) There was a higher frequency with fixation in the dark (Condition 3) than with fixation in the light (Condition 2) ($P<0\ 05$)

Correlation coefficients were also computed between experimental conditions for the 22 children who had successful trials in all 6 visual input conditions For the *uninterrupted durations*, there were no significant correlations between complete darkness (Condition 6) and the other 5 conditions ($r\leqslant +0\ 32$) The nystagmus durations under the 3 conditions in which visual fixation was possible (Conditions 1, 2, and 3) were all highly correlated with each other ($+0\ 63\leqslant r\leqslant +0\ 83$, $P<0\ 01$) and were independent of the conditions in which visual fixation was precluded (Conditions 4 and 5) ($r\leqslant +0\ 23$) The latter 2 conditions were highly correlated with each other ($r\geqslant +0\ 55$, $P<0\ 01$) Similar results obtained for the *total duration scores*, except that Conditions 1 and 3 were less strongly correlated ($P<0\ 05$) Similar results also obtained for the *total number of beats*, except that Condition 6 was highly correlated with Conditions 4 and 5 ($r>+0\ 78$, $P<0\ 01$)

## DISCUSSION

The main finding of this investigation is that a group of normal children show a significant reduction of post rotatory nystagmus duration and total number of beats under conditions in which visual fixation is precluded but in which light impinges on the retina The suppression of nystagmus duration is not as profound as that induced by visual fixation in the absence of light input, but the suppression of the total number of beats is the same in light without fixation as during fixation without light The greatest suppression of post-rotatory nystagmus occurs in those conditions which involve the *influence of both fixation and light Thus fixation* and light appear to have an additive effect in the suppression of vestibular nystagmus This effect does not depend on a positive correlation between the separate responses to light and fixation since the correlation analyses demonstrated that the subjects' responses to conditions permitting fixation were independent of their responses to conditions permitting light input but precluding fixation

There have been almost no systematic studies of vestibular nystagmus in normal children Allowing for differences in stimulation parameters, our post-rotatory nystagmus durations and frequencies in the dark are reasonably similar to the post-acceleratory nystagmus durations and frequencies reported by Tibbling (1969) for the same age group

While the nystagmus duration and the number of beats give an incomplete measure of vestibular activity (Tibbling, 1969), the post-acceleratory nystagmus duration does reflect the intensity of the stimulus (*Fluur & Mendel, 1969) In the* present investigation both the nystagmus duration and the total number of beats can be considered measures not of the total nystagmus intensity but rather of the *persistence* of nystagmus following an abrupt deceleration under the different conditions of visual input The results pertaining to the *frequency* of nystagmus beats help to understand the nature of the suppression of nystagmus duration and total number of beats in the presence of retinal stimulation with light The nystagmus frequency was significantly greater during the two conditions wh couraged ocular fixation than during

conditions, and there were no differences in frequency during the light without fixation conditions and complete darkness Greater frequency is a result of smaller slow phase amplitude or greater slow phase velocity or both While these parameters were not quantified in this study, it was clear from inspection of the nystagmograms that during ocular fixation (Conditions 2 and 3), slow phase amplitude was severely depressed This was not the case during light without fixation (Conditions 4 and 5) when the amplitudes tended to be similar to those recorded in complete darkness (Condition 6) Consequently, the frequency data verify the fact that the reduced *persistence* of nystagmus during the light without fixation conditions is not related to ocular fixation as is the case when a fixation object is provided [1] This suggests that vestibular nystagmus can be suppressed by at least two relatively independent mechanisms (1) a mechanism that is primarily oculomotor and does utilize ocular fixation, and (2) a mechanism that is primarily oculosensory and does *not* utilize ocular fixation

The former mechanism may be related to inhibitory cerebellar influences (Wolfe, 1969) The latter mechanism, in which light *per se* impinging on the retina causes the suppression f vestibular nystagmus, is obscure However, icular control of the vestibular nuclei is ntial to the generation of nystagmus in esponse to vestibular stimulation (Pompeiano, 1972) Also, light evoked discharges from lateral geniculate nucleus neurons can be altered by vestibular stimulation and connections from the vestibular nuclei to the reticular formation and from the reticular formation to the lateral geniculate nuclei have been described (Papaioannou, 1973) Therefore, it seems possible that mechanisms may exist whereby the effect of light on vestibular nystagmus is mediated by the reverse pathway, i e, from the lateral geniculate nuclei via the reticular formation to the vestibular nuclei Such a mechanism would provide additional stability of visual perception during motion

[1] Parenthetically, it can be seen that these young children did not fixate very well in Condition 1 when fixation was possible but not encouraged by a fixation object The frequency in Condition 1 was not greater than in Conditions 4 5, and 6

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## ZUSAMMENFASSUNG

Die Dauer, Gesamtzahl der Schläge und Frequenz des Postrotationen Nystagmus wurden an normalen jungen Kindern unter verschiedenen Zuständen der visuellen Leistungsaufnahme gemessen Nach der Rotation im Dunklen wurde die Wirkung von Fixierung ohne Licht, Fixierung im Licht und Licht ohne Fixierung auf den durch plotzliche Bremsverlangsamung hervorgerufenen Nystagmus studiert Die Dauer des Postrotationen-Nystagmus und die Gesamtzahl der Schlage wurden durch Fixierung ohne Licht und durch Licht ohne Fixierung bedeutend vermindert Diese Resultate wurden an Hand des Mechanismus mit dem vestibulärer Nystagmus unterdruckt wird besprochen

## REFERENCES

Colbert E G, Koegler, R R & Markham C H 1959 Vestibular dysfunction in childhood schizophrenia. *AMA Arch Gen Psychiat 1*, 600

Collins W E 1966 Vestibular responses from figure skaters *Aerosp Med 37*, 1098

— 1968 Special effects of brief periods of visual fixation on nystagmus and sensations of turning *Aerosp Med 39*, 257

Fluur, E. & Mendel L 1969 Relation between strength of acceleration and duration of postacceleratory nystagmus *Acta Otolaryng* (Stockh) *68* 127

Hood J D 1970 The clinical significance of vestibular habituation *Adv Otorhinolaryng 17*, 149

Marshall J E & Brown J H 1967 Visual-arousal interaction and specificity of nystagmic habituation. *Aerosp Med 38*, 597

Papaioannou J N 1973 Changes in the light-evoked discharges from lateral geniculate nucleus neurones in the cat, induced by caloric labyrinthine stimulation *Exp Brain Res 17*, 10

Pfaltz, C R & Piffko, P 1970 Studies on habituation of the human vestibular system *Adv Otorhinolaryng 17* 169

Pollack M & Krieger, H P 1958 Oculomotor and postural patterns in schizophrenic children *AMA Arch Neurol Psychiat 79* 720

Pompeiano, O 1972 Reticular control of the vestibular nuclei Physiology and pharmacology *Progr Brain Res 37* 601

Ritvo E R., Ornitz, E M, Eviatar, A, Markham C H, Brown M B & Mason A 1969 Decreased postrotatory nystagmus in early infantile autism *Neurology 19* 653

Tibbling L 1969 The rotatory nystagmus response in children *Acta Otolaryng* (Stockh) *68*, 459

Wendt G R 1951 Vestibular functions In *Handbook of experimental psychology* (ed S S Stevens) Wiley, New York

Wolfe J W 1969 Mesodiencephalic and cerebellar influences on optokinetic and vestibular nystagmus *Exp Neurol 25* 24

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# MALIGNANCY IN PLEOMORPHIC ADENOMA

## A Clinical and Microspectrophotometric Study

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*Abstract* A clinical study of carcinoma in pleomorphic adenoma based on the most extensive material described in the literature hitherto, strongly indicates that a malignant transformation of a primarily benign pleomorphic adenoma may occur and that the risk of such transformation increases with the age of the tumour (the preoperative duration of the tumour) A microspectrophotometric DNA analysis shows a difference in the DNA content between morphologically benign pleomorphic adenomas with short and with long preoperative duration of the tumour As cells from pleomorphic adenomas with a short preoperative duration contain the same diploid DNA value as normal parotid gland cells the pleomorphic adenomas with a longer preoperative duration are characterized by the occurrence of a small fraction of tetraploid cells in the population This small fraction of tetraploid cells may be of great importance for the velopment of malignancy in these tumours as it was d that the cases of carcinoma in pleomorphic adena also have a tetraploid (or near tetraploid) fraction cells It is tempting to suggest that the tetraploid cells of the carcinoma in pleomorphic adenoma have originated from the tetraploid cells occurring in benign pleomorphic adenomas with prolonged clinical course

The present clinical and microspectrophotometric study indicates the importance of an early operation even of the benign pleomorphic adenomas as the risk of malignant transformation of this type of tumour has been shown to increase with the preoperative duration of the tumour

In recent years many of the debated problems concerning the malignancy of pleomorphic adenomas (mixed tumours) have been solved Thus it has been shown that the concept of semimalignancy lacks justification and that the incidence of malignancy in pleomorphic adenoma is very low—about 1–3 % in large materials histologically classified according to modern nomenclature (Beahrs et al, 1957, Ackerman & del Regato, 1962, Eneroth, 1964, Eneroth et al., 1968)

It is still questioned, however, whether certain pleomorphic adenomas are primarily malignant or whether they become malignant at a later stage owing to a malignant transformation in a primarily benign pleomorphic adenoma Evans & Cruickshank (1970) pointed out that if primary malignant mixed tumours (pleomorphic adenomas) exist at all, they must be exceedingly rare and that there is no question that a malignant transformation of an initially benign tumour can occur The demonstration of a carcinomatous component in a benign pleomorphic adenoma and the demonstrated lack of pleomorphic adenoma structures in the metastases of these tumours argue in favour of the development of a carcinomatous component within the otherwise benign tumour (Eneroth et al, 1968). It has been discussed (Eneroth et al, 1968) whether this malignant change is related to the age of the tumour (preoperative duration of the tumour) This assumption however, has not yet been satisfactorily proved The reason is that malignancy in pleomorphic adenomas is very rare and therefore there is a lack of tumour materials of such a size that reliable conclusions concerning this question can be drawn

Table I *Case material microspectrophotometrically analysed*

| Histological features | Case no | Preoperative duration of the tumour (years) | Patients | |
|---|---|---|---|---|
| | | | Age (years) | Sex |
| Pleomorphic adenoma | 1 | 2/12 | 39 | ♀ |
| Pleomorphic adenoma | 2 | 1/12 | 52 | ♀ |
| Pleomorphic adenoma | 3 | 6/12 | 33 | " |
| Pleomorphic adenoma | 4 | 5 | 52 | " |
| Pleomorphic adenoma | 5 | 5 | 36 | ♂ |
| Pleomorphic adenoma | 6 | 6 | 55 | " |
| Carcinoma in pleomorphic adenoma | 7 | 16 | 69 | " |
| Carcinoma in pleomorphic adenoma | 8 | 15 | 59 | ♂ |

In order to ascertain whether the malignant transformation in a pleomorphic adenoma is dependent on the preoperative duration a very large material of parotid tumours has been studied clinically and cells from pleomorphic adenomas with and without malignancy have been analysed microspectrophotometrically

The importance of the time factor for malignant transformation in a benign pleomorphic adenoma can perhaps be elucidated by a microspectrophotometric DNA analysis, as increased nuclear DNA content has been shown to be a characteristic feature of malignant salivary gland tumour cells (Eneroth & Zetterberg, 1974)

For study of the influence of time on the nuclear DNA content in benign pleomorphic adenomas, microspectrophotometric DNA analyses were performed on cells from pleomorphic adenomas with different preoperative durations of the tumour and compared with the DNA values of cells from carcinoma in pleomorphic adenomas The reason for investigating the nuclear DNA content in pleomorphic adenomas with various preoperative durations of the tumour was the possibility to find a difference of nuclear DNA content between cells from tumours with short and long preoperative duration—a difference which could reveal an early stage of malignant transformation of the tumour before any histological features of malignancy can be seen

## MATERIAL AND METHODS

*The clinical study* was based on 623 pleomorphic adenomas of the parotid gland with or without malignancy, treated at the Department of Oto-laryngology, Karolinska Sjukhuset, during the period 1930–1964 Forty patients with carcinoma in pleomorphic adenoma registered and treated at Radiumhemmet, Karolinska Sjukhuset, during the period 1923–1972 have also been studied About 98 % of the patients underwent regular follow-up examinations for 5–40 years

*The microspectrophotometric study* was based on 8 pleomorphic adenomas with or without malignancy, treated at the Department of Oto-laryngology, Karolinska Sjukhuset, in the year 1971 The age of the tumour, defined as the preoperative duration of the tumour, was less than one year in 3 cases of pleomorphic adenoma, more than four years in 3 cases of pleomorphic adenoma, and more than 15 years in 2 cases of carcinoma in pleomorphic adenoma (Table I)

Immediately after the surgical removal of the tumours, imprint preparations were made from the fresh cut surface through the tumour tissue Haemacytometer glass slides were used The imprint preparations were immediately fixed in a freshly prepared mixture of ethanol and acetone (1 1) for 30 minutes at room temperature and thereafter stored in a refrigerator (+4 C) until the staining was performed

For the microscopic examination of tumours,

Table II *The relation between the preoperative duration of the tumour and the incidence of malignancy in pleomorphic adenomas treated 1950–64*

| Histological features | Preoperative duration of the tumour (Years) 0–4 | 5–9 | 10–14 | >15 | Total no of cases |
|---|---|---|---|---|---|
| Pleomorphic adenoma | 430 | 80 | 48 | 48 | 606 |
| Carcinoma in pleomorphic adenoma | 7 | 2 | 3 | 5 | 17 |
| Total | 437 | 82 | 51 | 53 | 623 |
| Incidence of malignancy (per cent) | 1 6 | 2 4 | 5 9 | 9 4 | |

routine histopathological techniques were employed

The DNA content of individual cell nuclei was determined after Feulgen staining by absorption measurements in a rapid scanning microspectrophotometer at 546 nm (Lomakka, 1965, Caspersson & Lomakka, 1970) The optimal condition of the acid hydrolysis in the Feulgen staining procedure was used as described by Eneroth & Zetterberg (1974)

Freshly prepared human lymphocytes from peripheral blood were used as control cells of the staining procedure on each occasion All measured values were expressed in relation to each staining control, which was given the value 2c, denoting the normal diploid DNA content

Stromal cells with elongated nuclei, lymphocytes and granulocytes could relatively easily be cognized in Feulgen-stained preparations and re not included in the measurements

## RESULTS

*Clinical study*

During the period 1950–64 the age of the tumour at the time of the histological diagnosis, i e the interval between the first symptom of the tumour and the operation (the preoperative duration), was registered in 623 patients with parotid tumours reclassified as pleomorphic adenomas with or without malignancy The histological features, the preoperative duration of the tumour as well as the influence of the duration on the incidence of malignancy in this material are shown in Table II It is seen that malignant histological structures appeared in 17 of the 623 tumours with pleomorphic adenoma features

The incidence of malignancy increases progressively with the preoperative duration of the tumour, from 1 6 % in tumours younger than 5 years to 9 4 % in tumours older than 15 years

As the incidence of malignancy in pleomorphic adenoma is very rare, the number of carcinomas in pleomorphic adenomas is comparatively small in relation to the large material of pleomorphic adenomas in the present series Therefore we have also made calculations of the incidence of malignancy in a larger tumour material of carcinoma in pleomorphic adenoma treated at Radiumhemmet during the period 1923–72 This larger material of 40 patients with

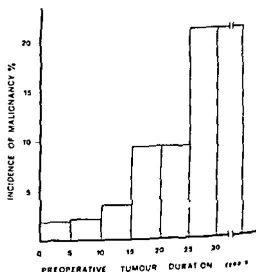



*Fig 1* Histogram showing the relation between the preoperative duration of the tumour and the incidence of malignancy in pleomorphic adenomas.

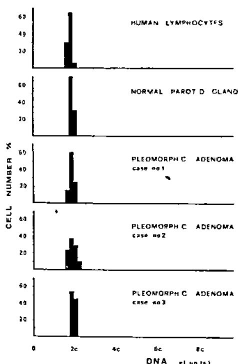



*Fig 2* Histograms of nuclear DNA quantity (Feulgen-positive material) of individually analysed cell nuclei in the rapid scanning microspectrophotometer Five different types of cell were analysed human lymphocytes from peripheral blood (staining controls), cells from a normal parotid gland and cells from three cases of pleomorphic adenoma with a preoperative duration less than one year (Cases 1, 2 and 3) Each histogram is based on the microspectrophotometric analysis of between 50 and 75 randomly selected cells The DNA values are expressed in relation to the staining control (human lymphocytes) which was given the value 2c

carcinoma in pleomorphic adenomas has been related to the aforementioned material of pleomorphic adenomas from the period 1950–64 The results of the calculations of the incidence of malignancy in this large material as a function of the preoperative duration of the tumour are illustrated in a histogram (Fig 1) From this histogram it is seen still more emphatically that the incidence of malignancy in pleomorphic adenomas increases progressively with longer preoperative duration of the tumour

The mean age of the 40 patients with carcinoma in pleomorphic adenoma was 52 2 years (range 23–83 years) There was no significant difference between the mean age of patients whose tumours were of less than 5 years' duration (mean age 51 6 years) and those whose tumours were of more than 5 years' duration (mean age 52 6 years)

The observed preoperative duration of tumours ranged from a few months up to 55 years (mean 11 2 years) It should, however, be noted that the duration may have been longer than the patients themselves have stated

### *Microspectrophotometric study of nuclear DNA content*

In order to investigate whether the occasional clinical malignancy in aging pleomorphic adenoma is associated with any changes of the cytochemical properties of cells from older tumours,

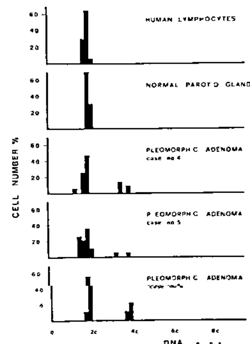



*Fig 3* Histograms of nuclear DNA quantity (Feulgen positive material) of individually analysed cell nuclei in the rapid scanning microspectrophotometer Five different types of cell were analysed human lymphocytes from peripheral blood (staining controls), cells from a normal parotid gland and cells from three cases of pleomorphic adenoma with a preoperative duration more than five years (Cases 4, 5 and 6) Each histogram is based on the microspectrophotometric analysis of between 50 and 75 randomly selected cells The DNA values are expressed in relation to the staining control (human ' which was given the value 2c

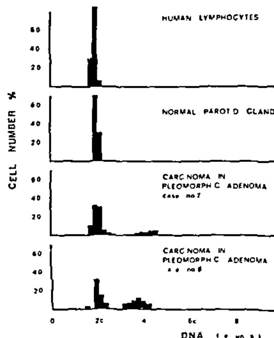



*Fig 4* Histograms of nuclear DNA quantity (Feulgen positive material) of individually analysed cell nuclei in the rapid scanning microspectrophotometer Four different types of cell were analysed human lymphocytes from peripheral blood (staining controls) cells from a normal parotid gland and cells from two cases of carcinoma in pleomorphic adenoma with a preoperative duration of 15 and 16 years respectively (Cases 7 and 8) Each histogram is based on the microspectrophotometric analysis of between 50 and 75 randomly selected cells The DNA values are expressed in relation to the staining control (human lymphocytes) which was given the value 2c

nuclear DNA content was measured in individual pleomorphic adenoma cells of tumours with short and long preoperative duration Fig 2 illustrates microspectrophotometric data of randomly selected Feulgen stained nuclei of imprint preparations from three cases of pleomorphic adenoma (cases 1, 2 and 3) with short preoperative duration (< one year) (cf Table I) As control cells of the Feulgen staining procedure human lymphocytes from peripheral blood were used The average DNA content of the lymphocytes was given the value 2c denoting the normal diploid DNA content Each measured value was related to its staining control, i e to the human lymphocytes stained together with the measured cell and expressed in the 2c lymphocyte units As is evident from Fig 2, all three cases of pleomorphic adenoma with short preoperative duration of the tumours (cases 1, 2 and 3) exhibited the same amount of DNA as normal parotid gland cells and lymphocytes namely the normal diploid content 2c However, as is seen in Fig 3, in the three cases of pleomorphic adenoma with long preoperative duration, i e more than 5 years (cases 4 5 and 6), an additional fraction of the cells varying from 10% to 30% of the population exhibited DNA values around 4c, i e twice the normal diploid value This could either represent diploid cells in the premitotic phase (G2 phase) of the cell cycle or cells with a tetraploid (or near tetraploid) chromosome number In the light of the very low mitotic index (less than 0.01%) which was observed in all these 6 cases of pleomorphic adenoma, it can be concluded that the cells with DNA content of 4c represent tetraploid (or near tetraploid) cells It is therefore of great interest to note that the 2 cases of carcinoma developed in a pleomorphic adenoma (cases 7 and 8) also show a fraction of cells in the tetraploid region i e around 4c relative units of DNA (Fig 4) This fraction contains from 18% (case 7) to 45% (case 8) of the cells Since these 2 cases of carcinoma in pleomorphic adenoma contain a mixture of relatively small benign adenoma cells and relatively large malignant carcinoma cells it is likely that the cells with nuclear DNA contents around 2c represent the adenoma cells and the cells with DNA values around 4c represent the carcinoma cells

## DISCUSSION

Although many of the problems concerning the malignancy of pleomorphic adenoma have now been answered, there is still a considerable uncertainty concerning the question whether a pleomorphic adenoma is a primarily benign type of tumour which can become malignant or whether carcinoma in pleomorphic adenoma is a primarily malignant type of tumour The probability of malignant transformation of an initially benign tumour has been revealed by a

stological study of primary tumours and metastases (Moberger & Eneroth, 1968) A malignant ansformation of a benign pleomorphic adenoma has also been suggested by the clinical atures, as many of these cases had a long clinical ourse compared to other malignant salivary and tumours In a large series of parotid tumours (Eneroth et al, 1968) the mean interval etween the first evidence of tumour and histological verification (the preoperative duration) inged from 3 7 to 6 4 years in other types of alignant tumours, such as adenoid cystic carcinoma, acinic cell carcinoma and mucoepidermoid carcinoma In carcinoma in pleomorphic lenoma this interval was 9 years There are ccasional reports in the literature of cases with very long preoperative duration of the tumour >20 years) and with a short survival time after ie operation, which indicates that malignant ansformation of the pleomorphic adenoma annot be regarded as improbable

In an attempt to elucidate the hypothesis that ie risk of malignant transformation in a pleomorphic adenoma increases with the preoperative duration of the tumour a comparison was iade between this duration and the incidence f malignancy in pleomorphic adenoma in a irge tumour material The results from the pres nt clinical study strongly indicate that malignant transformation of a pleomorphic adenoma a function of time

The earlier uncertainty in evaluating the malignancy of pleomorphic adenomas appears from he literature, since some authors divide these umours into benign, semimalignant and ma gnant, others denote the whole group as solely enign, solely semimalignant or solely malignant Correlation studies between histological nd clinical features showing that this type of umour should be divided into a benign and a nalignant group (Eneroth, 1964) are also supported by the microspectrophotometric DNA inalysis of the present study

The microspectrophotometric data obtained n the present study show that cells from pleomorphic adenoma contain the diploid DNA value, i e these tumour cells have identical DNA values to those of normal parotid gland cells, but they clearly differ from the cells of malignant salivary gland tumours described by Eneroth & Zetterberg (1974)

The results from the clinical study indicate the importance of the time factor for malignant transformation of a pleomorphic adenoma and in order to study whether the time factor also influences the nuclear DNA content of cells from pleomorphic adenomas, a microspectrophotometric DNA analysis was performed on cells from pleomorphic adenomas with different preoperative duration This study showed that there was a difference in the DNA content between histologically benign pleomorphic adenomas with short and long preoperative duration As cells from pleomorphic adenomas with a short preoperative duration (< one year) contain the diploid DNA value, the pleomorphic adenomas with a longer preoperative duration (>5 years) are characterized by the occurrence of a small fraction of tetraploid cells (or near tetraploid cells) in the population

However, the majority of the cells still have the diploid DNA value characteristic of normal parotid gland cells Nevertheless this small frac tion of tetraploid (or near tetraploid) cells may be of great importance for the development of malignancy in these tumours as discussed above For it was found that the 2 cases of carcinoma in pleomorphic adenoma of the present study (cases 7 and 8) also have a tetraploid (or near tetraploid) fraction of cells, probably representing the malignant cells, in addition to the cells with normal diploid DNA values These two malignant tumours (carcinoma in pleomorphic adenoma) thereby differ from other malignant salivary gland tumours which are characterized by the existence of aneuploid stemlines in the hyperdiploid region (Eneroth & Zetterberg, 1974) It is tempting to suggest that the tetraploid malignant cells have originated from the tetraploid cells of the benign p' adenomas, which may be more sus malignant transformation than counterparts This is supported by an aberrant chromosome co

the malignant transformation of cells, as indicated by Levan & Biesele (1958) in their experiments with embryonic mouse skin and cultures Of particular relevance to the present work was their observation that the first cytological event in the transformation process was a shift in the chromosome number from diploidy to tetraploidy Pleomorphic adenomas containing tetraploid cells might thus represent a potentially malignant tumour

A wait-and see policy nevertheless exists regarding the treatment of parotid tumours, perhaps due to earlier statements in the literature that operation of parotid tumours is unnecessary (McFarland, 1933, 1942) and to the fact that the great majority—about 80% of parotid tumours—are benign (Eneroth, 1971)

The present clinical study has, however, *shown that the risk of malignant transformation* of a benign pleomorphic adenoma increases with the age of the tumour, and also the present microspectrophotometric analysis has shown nuclear DNA characteristics (tetraploid cells) in pleomorphic adenoma with a prolonged clinical course, which might be a preliminary stage of a malignant transformation

Thus the results of the present clinical and microspectrophotometric studies indicate the importance of an early operation of all parotid tumours—even the benign pleomorphic adeno-

s


## ZUSAMMENFASSUNG

Wie die vorliegende klinische Untersuchung an Karzinomen in pleomorphen Adenomen, die auf dem grossten bisher in der Literatur beschriebenen Material basiert, entnehmen lasst, ist es sehr wahrscheinlich, dass bei einem ursprunglich benignen pleomorphen Adenom eine maligne Transformation eintreten kann und dass die Gefahr einer solchen Transformation mit dem Alter des Tumors, d h mit der präoperativen Dauer des Tumors, wächst Wie die mikrospektrophotometrische DNS-Analyse zeigt, besteht ein Unterschied im DNS-Gehalt zwischen morphologisch benignen pleomorphen Adenomen von kurzer und von langer präoperativer Dauer des Tumors Während Zellen von pleomorphen Adenomen von kurzer präoperativer Dauer den gleichen diploiden DNS-Betrag wie normale Parotiszellen enthalten, ist es für pleomorphe Adenome mit längerer präoperativer Dauer charakteristisch, dass bei ihnen auch ein gewisser (kleiner) Bruchteil an tetraploiden Zellen auftritt Dieser Bruchteil an tetraploiden Zel[...] kann eine grosse Bedeutung in der Entwicklung d[...] Malignität dieser Tumoren haben, da *festgestellt werd[...]* konnte, dass in Fallen von Karzinomen in pleomorph[...] Adenomen auch ein gewisser Bruchteil von tetraploide[...] bzw im tetraploiden Bereich liegenden Zellen vorlie[...] Es ist daher naheliegend sich vorzustellen, dass d[...] tetraploiden Zellen der Karzinome in pleomorph[...] Adenomen aus den tetraploiden Zellen hervorgegang[...] sind, *die in benignen pleomorphen Adenomen [...]* prolongiertem klinischen Verlauf auftreten

Die vorliegende klinische und mikrospektrophot[...] metrische Untersuchung unterstreicht, wie wichtig es [...] auch benigne pleomorphe Adenome fruhzeitig zu operi[...] ren, da, wie gezeigt werden konnte, die Gefahr ein[...] malignen Transformation dieses Tumortyps mit d[...] präoperativen Dauer des Tumors zunimmt


## REFERENCES


Ackerman, L V & del Regato, J A 1962 *Cance[...] Diagnosis, Treatment, and Prognosis* 3rd ed. C [...] Mosby Company, St Louis

Beahrs, O H, Woolner, L B, Kirklin, J W & Devin[...] K D 1957 Carcinomatous transformation of mix[...] tumours of the parotid gland *Arch Surg (Chic)* 7[...] 605

Caspersson, T & Lomakka, G 1970 *Introduction [...] quantitative cytochemistry* (ed G Bahr and G Wi[...] vol 2 Academic Press, New York

Eneroth, C-M 1964 Histological and clinical aspe[...] of parotid tumours *Acta Otolaryng* (Stockh) Sup[...] *191*

— 1971 Salivary gland tumors in the parotid gla[...] submandibular gland and the palate region. *Cancer* [...] 1415

Eneroth, C-M, Blanck, C & Jakobsson P Å 19[...] Carcinoma in pleomorphic adenoma of the paro[...] gland *Acta Otolaryng* (Stockh) 66, 477

Eneroth, C-M & Zetterberg, A 1974 Microspectro[...] photometric DNA analysis of malignant saliva[...] gland tumours *Acta Otolaryng* (Stockh) 77, 289

[...] A H 1970 *Ei[...] [...]inden Co[...]*



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# DECONGESTION OF NASAL MUCOUS MEMBRANES BY ORAL MEDICATION IN ACUTE RHINITIS

*A Rhinomanometric Study to Demonstrate Synergism between Antihistamines and Adrenergic Substance*

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(Received November 12, 1973)

*Abstract* Clinical trials of antihistamines combined with adrenergic substances showed a decongestive effect on nasal mucosal congestion due to acute rhinitis Double blind technique combined with objective rhinomanometry over 9 hours in each individual trial showed complete absence of placebo effect, the results were therefore conclusive Tests on 85 subjects with acute rhinitis as well as tests on 50 normal subjects with histamine provoked nasal blocking support the idea that an antihistamine and an adrenergic substance show synergism with regard to nasal decongestion Of the drugs tested, clemastine combined with phenylpropanolamine gave the best result

Decongestion of the nasal mucous membranes by oral medication is widely practised in patients with nasal allergy Antihistamines or antihistamines combined with an adrenergic substance, assuming a synergism between the two types of drug, are the preparations most commonly used It is practically unknown however, whether this therapy is efficacious in congestion due to infection The aim of the investigation was to ascertain whether oral medication might be of value in nasal blocking due to infection as well as in allergic congestion, and whether the synergism between an antihistamine and an adrenergic substance occurring in allergic nasal decongestion is a general rule In an investigation published in 1964 Aschan demonstrated synergism between antihistamine and adrenergic substance in histamine provoked nasal congestion This was the reason for including similar experiments in the present investigation for purpose of comparison

## MATERIAL AND METHODS

Altogether 85 subjects with nasal blocking due to acute rhinitis were included in the first part of the investigation The tests were performed 2–3 days after the onset of the illness, and only individuals free from complications were included The tests took place at a time of year when pollen allergy could be excluded as a source of error

For the histamine provoked nasal blocking a total of 50 apparently healthy subjects were selected Some took part in two experiments, the second after an interval of at least 4 weeks

The nasal patency was measured by repeated rhinomanometric recordings, using the method described by Aschan et al (1956 and 1958) Recordings were made at intervals of 30–50 min over a period of 9 hours, and it was possible to trace the patency and changes in patency fairly well (see Figs 1 and 2) At each observation the airflow was recorded and calibrated to litres per min at a pressure gradient of 10 mm of water between the nostrils and oropharynx For this pressure gradient an airflow of 30–40 litres per min may be regarded as normal A lesser airflow indicates nasal congestion

Double blind investigations were carried ~ for all drugs tested, and the key mizing list was opened and · rhinomanometric findings only evaluated

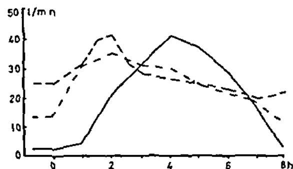



*Fig 1* Rhinomanometric changes in nasal patency in 3 subjects with nasal blocking due to acute rhinitis Note the variations in patency before drug administration at 0 All test with active drug

In the series with acute rhinitis the degree of nasal blocking before drug administration varied greatly from mild blocking to almost complete blocking of the nose, as is shown by the three positive experiments in Fig 1 For this reason the final evaluation of an experiment was simplified to either positive as in Fig 1, or negative With complete or nearly complete blocking the rhinomanometric method does not work If, for example, a subject with initial rhinomanometric values as in the lower curve in Fig 1, did not respond, the rhinomanometric assessment ought to have been '?', but might of course also have been 'negative' Because the double blind technique was used, such individual experiments had o be included

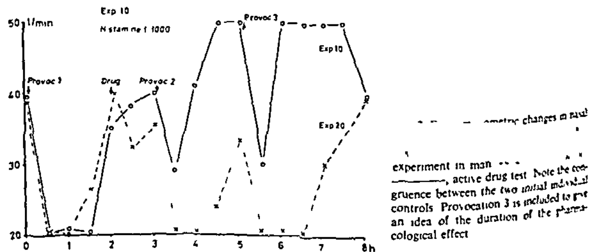
Concerning the histamine experiments, each provocation involved the spraying of 1 5–2 ml histamine (1 1000) into each nostril An initial provocation served as individual control The subsequent histamine provocations after drug administration were performed to ascertain how long the drug action, if any, persisted In the experiments in Fig 2, the dotted line represents a negative rhinomanometric finding the unbroken line a positive result In fact both experiments were performed on one and the same subject after an interval of several months and could be termed a double blind cross-over experiment The first control histamine provocation in the two experiments in Fig 2 also illustrates the reproducibility in the rhinomanometric method

## RESULTS

In an initial series 30 subjects with acute rhinitis were tested with '5031' (=15 mg N hydroxyethyl promethazine chloride + ephedrine 10 mg) The results are shown in Table I

A positive rhinomanometric result was obtained in 10 out of 15 tests using the active drug No placebo effect whatsoever was observed or recorded by this objective method of assessment The significance of the 10 out of 15 positive findings of nasal decongestion in acute rhinitis using 5031, compared with the placebo group must therefore be regarded as very high and conclusive

In a second series (Table II) consisting of 15 subjects with acute rhinitis another antihista




... ometric changes in nasal ... experiment in man ... ———, active drug test Note the congruence between the two initial individual controls Provocation 3 is included to give an idea of the duration of the pharmacological effect

Table I *5031 Acute rhinitis*

| Drug number | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Judgement of rhinomanometric recording | + | | ? | − | + | − | − | + | + | − | | + | ? | + | |
| Placebo | | | × | × | | × | × | | | × | × | | × | | |
| 5031 | × | × | | | × | | | × | × | | | × | | | |

| Drug number | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 | 25 | 26 | 27 | 28 | 29 | 30 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Judgement of rhinomanometric recording | ? | | + | − | − | + | + | + | | | | ? | | | ? |
| Placebo | | × | | × | × | | | | × | × | × | | × | × | |
| 5031 | × | | × | | | × | × | × | | | | × | | | × |

mine 'HS 592' (clemastine 1 0 mg) was tested alone and in combination with another adrenergic substance, HSP 525 I (clemastine 1 0 mg + 50 mg phenylpropanolamine) The antihistamine was chosen because it shows extremely little or no sedative effect, and the adrenergic substance also shows fewer side effects than does ephedrine No placebo effect was recorded, and there was no effect of antihistamine alone, but the combined drug, HSP 525 I, gave positive nasal decongestion in 4 out of 5 tests in these subjects with acute rhinitis

After these promising results, and with the question of synergism between antihistamine and adrenergic substance in mind, the logical course was to design a new series testing all components separately and combined The third series comprised 20 subjects with acute rhinitis tested with HS 592 (clemastine 1 0 mg), HSP 525 II (clemastine 1 0 mg + phenylpropanol amine 30 mg), and PPA (phenylpropanolamine 30 mg) The results are shown in Table III There was still no placebo effect, positive responses were noted in 2 out of 5 cases for both the antihistamine and the adrenergic substances alone, and this time there was a positive response to *the combined drug in all five cases* This series thus conclusively proves both the feasibility of oral decongestion in acute rhinitis, and the hypothesis concerning synergism between antihistamine and adrenergic substance in connexion with this state

As, however, the blood concentration of phenylpropanolamine could be expected to persist for a much briefer period than that of clemastine, still better results might conceivably be obtained if the effect of PPA could be pro longed and its blood concentration curve thus

Table II *HSP 525 I Acute rhinitis*

| Patient number | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Judgement of rhinomanometric recording | − | | | | | | | | | | | | | | |
| Placebo | | | | | | | | | | | | | | | |
| HS 592 | | | | | | | | | | | | | | | |
| HSP 525 I | | × | | | | | | | | | | | | | |

Table III *HSP 525 II Acute rhinitis*

| Patient number | 21 | 22 | 23 | 24 | 25 | 26 | 27 | 28 | 29 | 30 | 31 | 32 | 33 | 34 | 35 | 36 | 37 | 38 | 39 | 40 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Judgem^nt of rhinomanometric recording | + | + | − | + | − | − | + | − | + | − | + | + | − | − | − | − | + | − | − | |
| Placebo | | | × | | | × | | | | | | | × | | × | | | | | |
| HS 592 | | | | × | × | | | | | | | × | | × | | | | | × | |
| HSP 525 II | | × | | | | | × | | × | | × | | | | | | | | | × |
| PPA | × | | | | | | | × | | × | | | | | | × | × | | | |

brought to coincide more closely with that of clemastine

The final series of tests on acute rhinitis consisted of 20 subjects The results are shown in Table IV, where HS 592 stands for clemastine 1 0 mg, PPA for phenyl propanolamine 50 mg retarded, and HSP 525 A for 50 mg PPA retarded with clemastine 1 0 mg There was no placebo effect and no effect of antihistamine alone The tests with both the combined preparation and the retarded phenylpropanolamine were all positive The decongestive effect of the combined drug and of the adrenergic substance on nasal blocking due to acute rhinitis is unequivocal Concerning the idea of synergism, the above-mentioned variation in patency in the subjects with rhinitis (see Fig 1) and the small umber of subjects in this series do not permit antitative comparisons between HSP 525 A nd PPA retarded However, there is no evidence against the hypothesis of synergism

Concerning the histamine experiments, the assessment of each individual experiment is explained in Fig 2 Three series were run, largely following the pattern of the experiments on acute rhinitis

In the first series 10 subjects were tested with placebo and with HSP 525 I (clemastine 1 0 mg with 50 mg phenylpropanolamine) There was no placebo effect, and the active drug was positive in all 5 cases

In a second series of 20 subjects HS 592 (clemastine 1 0 mg) PPA (30 mg phenylpropanolamine), and HSP 525 II were tested There was no placebo effect The antihistamine alone was positive in one out of 5, PPA positive in 5 out of 5, and HSP 525 II was positive in 3 out of 5 subjects

In the last series of experiments on another 20 test persons the phenylpropanolamine used was '50 mg retarded', HS 592 and HSP 525 A, as in Table III above Again as throughout the whole investigation, there was no placebo effect. With the antihistamine alone one test out of 5 was positive, with phenylpropanolamine retarded 4 out of 5 were positive, and for the antihistamine combined with the retarded phenylpropanolamine all five were positive

Table IV *HSP 525 A Acute rhinitis*

| Patient number | 21 | 22 | 23 | 24 | 25 | 26 | 27 | 28 | 29 | 30 | 31 | 32 | 33 | 34 | 35 | 36 | 37 | 38 | 39 | 40 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Judgement of rhinomanometric recording | | | | | | | | | − | + | − | + | − | − | + | | + | + | − | |
| Placebo | | | | | | | | | | | | | × | | | | | | | |
| HS 592 | | | | | | | | | | | × | | | × | | | | | × | |
| HSP 525 A | | | | | | | | | | | | | | | | | | | | |
| PPA | | | | | | | | | | | | × | | | × | | × | | | |

The histamine series thus supported the hypothesis of synergism between antihistamine and adrenergic substance with regard to nasal decongestion, even with this type of experimental nasal blocking in man As can be seen from the typical experiment illustrated in Fig 2, the active drug seemed to be effective for at least $5^1/_2$ hours after administration

## DISCUSSION

In clinical praxis nose drops are used for decongestion of the nasal mucous membranes no matter what the cause of the congestion Topical administration of drugs has some drawbacks, however Nose drops used for more than one week often cause remote reactions, and the patient becomes 'hooked' on his nose drops This situation has not yet been reported in connexion with oral medication Further, with topical administration, drugs producing maximum therapeutical responses on the nano- or microgram blood level must be given in concentrations as high as 5 to 10 mg/ml As both nose drops and drugs given by mouth to produce nasal decongestion will act via the nasal blood vessels it would seem preferable to use the oral route, especially for long-term treatment In clinical praxis it is as a rule desirable also to achieve decongestion of adjoining mucous membranes derived from the upper airways—the mucosa within the sinuses and their ostia, the Eustachian tube, and the middle ear The chances of reaching these structures must be larger if the drug is distributed via the blood stream than if it is administered locally This is particularly important when blocking is due to inflammatory causes

Clinical testing in man has several short comings The subjects with nasal blocking due to acute rhinitis were very carefully selected and represent only one type of inflammatory nasal blocking The variations in degree of nasal blocking before drug administration represent another source of error however, with the complete absence of placebo effects, the results must be regarded as conclusive The drug combination marked 5031 had a decongestive effect in 10 out of 15 tests The combination clemastine with phenylpropanolamine showed still better results, with 14 positive tests of 15, disregarding some galenic differences in the composition of the drug tested (HSP 525 I, HSP 525 II, and HSP 525 A) The significance of the results is proved by the thirty placebo tests, all of which were negative The objective rhinomanometric technique used here shows that oral administration is the method of choice for obtaining nasal decongestion even when the nasal obstruction is due to inflammation

A series of studies by Jackson (1971), Bentley & Jackson (1970) and Hall & Jackson (1968), on nasal patency, the sinuses, and the Eustachian tube in dogs, indicate that the mucous membranes in the different organs behave rather similarly from a pharmacological point of view From their experiments it is evident that the nasal blood flow is regulated via nervous and pharmacological pathways Dahlström & Fuxe (1965) have demonstrated the adrenergic innervation of the nasal blood vessels They have also shown that no adrenergic fibres are present round the glands of the nasal mucosa in their animals, an interesting fact often neglected in connexion with nasal patency Cauna & Hinderer (1969) have demonstrated by electron microscopy a dense innervation of the blood vessels in the human nasal mucosa All the investigations quoted are of interest because they in different ways indicate that the decongestion obtained in the nasal mucosa by oral medication also occurs in adjoining structures, thus extending the indications for the use of such drugs

The synergism between antihistamine and adrenergic substances in allergic nasal congestion is documented (McLaurin et al (1961), Pullen & Montgomery (1963), Aschan (1964), and others) Bentley & Jackson (1970) in experiments on dogs found a synergistic effect of several antihistamines and epinephrine upon histamine-provoked nasal blocking The results of the experiments on acute rhinitis and of the with histamine-provoked nasal co man support the idea The reason

clemastine in greater detail in this investigation was that it is known to produce little or no sedative effect Phenylpropanolamine was chosen because of certain undesirable side-effects of ephedrine, particularly in elderly subjects

The value of rhinomanometry in investigations of this type must be strongly emphasized The reproducibility of the individual controls in the histamine provocation shown in Fig 2 is one way of illustrating this The results of Büsser & Schibli (1973), who used a somewhat different technique, provide further confirmation (see also Bühlmann, 1973) The complete absence of placebo effect in the double-blind series is further proof that rhinomanometry is the method of choice in clinical trials of this type

## ZUSAMMENFASSUNG

Klinische Versuche mit Antihistaminika in Kombination mit adrenergischen Substanzen ergaben einen dekongestiven Effekt bei Schwellung der Nasenschleimhaut aufgrund akuter Rhinitis Doppelblindtechnik verbunden *mit objektiver Rhinomanometrie* über einen Zeitraum von 9 Stunden bei jedem einzelnen Versuch zeigte das vollständige Ausbleiben des Placebo-effekts, die Resultate erwiesen sich daher als unverlassig Versuche, durchgefuhrt an 85 Patienten mit akuter Rhinitis, sowie Versuche mit 50 normalen Versuchspersonen mit histamin-provozierter Blockade der Nase unterstutzten die Annahme, dass Antihistamin zusammen mit einer adrergischen Substanz Synergismus mit Wirkung auf die le Dekongestion zeigt Von den untersuchten Präpaten erzielte man die besten Ergebnisse mit einer Kombination von Clemastine 1,0 mg und Phenylpropanolamine 50 mg

## REFERENCES

Aschan, G 1964 Experimentell klinisk prövning av H- [illegible]

Aschan, G , Drettner, B & Ronge, H 1956 *En objektiv metod for mätning och registrering av näsandnings-motståndet* *Svensk Otolaryng For Forh* Hälte 2

— 1958 A new technique for measuring nasal resistance to breathing illustrated by the effects of histam[ine] and physical effort *Ann Acad Sci Upsaliensis 2* 1[illegible]

Bentley, A J & Jackson, R T 1970 Changes in patency of the upper nasal passage induced by his[ta]mine and antihistamines *Laryngoscope 80* 1859

Buhlmann, A A 1973 Measure of the breathing res[ist]ance in the nasal passage *Trans X Int World Co[ngr] Otorhinolaryngol* Excerpta Medica To be publish[ed]

Büsser, E & Schibli, R A 1973 Rhinomanometrie M[e]thodik und Normalwerte *Deutsch Med Wschr 98* [illegible]

Cauna, N 1970 Electron microscopy of the nasal v[as]cular bed and its nerve supply *Ann Otol 79* 443

Cauna, N & Hinderer, K H 1969 Fine structure [of] blood vessels of the human nasal respiratory muco[sa] *Ann Otol 78*, 865

Dahlstrom A & Fuxe, K 1965 The adrenergic inner[va]tion of the nasal mucosa of certain mammals *A[cta] Otolaryng* (Stockh ) *59*, 65

Hall, L J & Jackson, R T 1968 Effects of alpha [and] beta adrenergic agonists on nasal blood flow *A[nn] Otol 77*, 1120

Jackson R T 1971 Pharmacological mechanisms in [the] Eustachian tube *Ann Otol 80*, 313

McLaurin, J W , Shipman, W F & Rosedale Jr [illegible] 1961 Oral decongestants A double blind compar[ative] study of the effectiveness of four sympathomim[etic] drugs, objective and subjective *Laryngoscope 71* [illegible]

Pullen II, F W & Montgomery W W 1963 Compa[ra]tive evaluation of oral decongestants *Arch Otolar[yng]* (Chic ) *77*, 10

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# ULTRASTRUCTURE OF THE EPITHELIUM IN ATROPHIC RHINITIS

*Scanning Electron Microscopic Studies*

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(Received October 29, 1973)

*Abstract* Biopsies from nasal mucosa of patients with atrophic rhinitis were studied in the scanning electron microscope A keratinized squamous epithelium, resembling skin, is seen over large areas Desquamation of horny cells is pronounced and abnormal, with large desquamated scales forming the basis for the well known crusts Openings from submucosal glands and goblet cells are very infrequent The small amount of mucus is dehydrated in situ, because of the lack of cilia

The great depth of focus of scanning electron microscopy (SEM), its wide range of magnifications, and relatively great resolving power, make it a logical tool for the observation of the delicate structures of the surface of the human nasal mucosa Within the last few years, several papers have appeared dealing with S E M studies of the normal nasal mucosa and the nasal mucosa from patients with perennial rhinitis (Lenz, 1972*a*, *b*, 1973, Mygind & Bretlau, 1973, 1974) In addition Lenz (1972*c*) studied the squamous epithelium in the anterior part of the nasal cavity and described the normal desquamation process of the epithelial cells In the present study the fine-structures of the horny cells and their spatial relationship within the squamous epithelium in atrophic rhinitis have been investigated An attempt has been made to correlate these observations with information available from transmission electron microscopic investigations (Mygind et al, 1974)

## MATERIAL AND METHODS

Six adult patients with pronounced atrophic rhinitis were examined For a longer period of time they had all suffered from crust-formation in the nose and they had only been treated by conservative means Biopsies of a size of 1½–3 mm were taken with a small forceps without local anaesthesia They were taken from the lower edge of the inferior turbinate ½–1 cm behind the anterior edge as well as from other places in the nasal cavity

For comparison purposes, biopsies were also obtained from the anterior part of the nasal cavity, at the borderline to the skin, in 3 normal persons

As glutaraldehyde causes epithelium to adhere to the forceps, the biopsies were loosened from the forceps in 0 9 % sodium cloride and immediately thereafter transferred to a cold 5 % solution of glutaraldehyde, buffered with a 0 03 M sodium cacodylate (pH 7 4) After 24 hours fixation the biopsies were stored in the buffer After dehydration in ethyl alcohol the specimens were transferred to benzene After freeze drying as described by Nørrevang & Wingstrand (1970), the specimens were mounted on stubs, and a conduction coat of gold was deposited in vacuum The specimens were studied in a C
Stereoscan S2



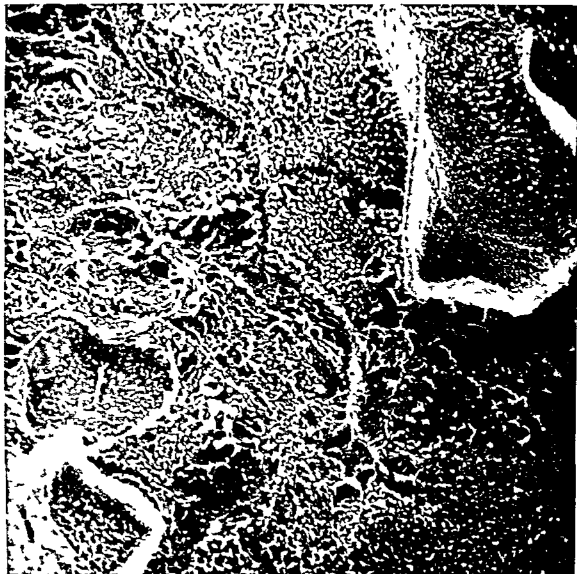
*Fig. 1.* Low-power magnification of nasal biopsy from patient with atrophic rhinitis. The surface of the mucous membrane is covered by irregular scales (×42)

## RESULTS

The surface-topography of the epithelium is revealed in considerable detail in the scanning microscope, which also gives a striking three-dimentional appearance. At low power magnification the atrophic mucosa looks highly abnormal, with the appearance of "reptile skin" with numerous scales, irregularly arranged and of unequal size (Fig. 1). At higher magnification one can see horny cells desquamate in the same manner as the horny cells in the anterior part of the nasal cavity, i.e. by initial elevation of the peripheral part of the cell (Fig. 2). However, in the atrophic mucosa it is much more common to see several cells loosen *in toto*, forming large scales (Fig. 3) or irregular dome-like shapes (Fig. 4). This suggests that the cells are quite flexible. Even though the horny cells are extremely thin, no examples of torn or fragmented cells were seen.

The horny cells in the skin of the anterior part of the normal nasal cavity are polygonal or hexagonal in shape and have smooth, straight intercellular junctions (Fig. 5). One frequently encounters the junction of three cells at the corners of the contiguous hexagons. The cell-surface is not smooth, but covered by long, branching folds.

Whereas the picture of the normal nasal mucosa is quite different from this (Mygind & Bretlau, 1973, 1974), the atrophic nasal mucosa even in the posterior part of the nose has several similarities to the normal skin; only minor differences are seen. The horny cells in the atrophic mucosa may be polygonal in shape as the cells in the skin, but they are often more round sh. and it may be difficult to discern the cell borders



*Fg 2* Surface of the atroph c mucous membrane Med um power magnif cat on In the upper right corner is seen a horny cell w th elevat on of its peripheral part, i e a normal desquamat on process The surface-struc tures of the cells are scat ered short, microvillous l ke project ons ( 1 800)

(Fig 2) In contrast to the horny cells in the normal skin where long branching folds are the dominant surface structure the cell surfaces of the atroph c mucosa are mostly covered by short microvillous like projections (Fig 2) These projections may be remnants of desmosomes On the whole the surface-structures of the atrophic mucosa are much shorter and more scattered than in the normal mucosa (Lenz, 1972*a* Mygind & Bretlau 1973) Actual microvilli (1 2 $\mu$m long structures) are not seen in the squamous epithelium

In the specimens investigated from patients with atrophic rhinitis the dominant type of epithelium was a horny squamous epithelium, found in 4 out of 6 patients In one biopsy mally looking cilia were seen In nearly hal biopsies areas were observed of

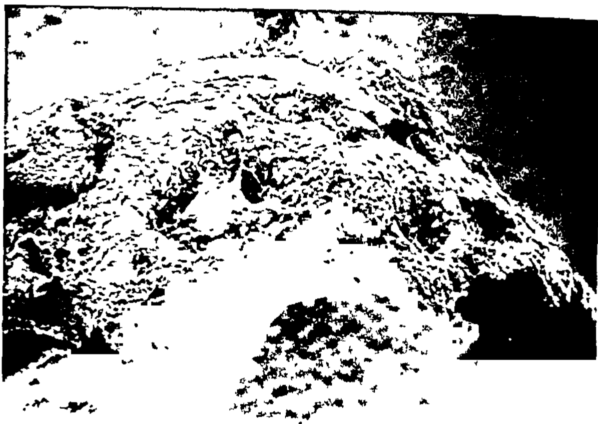


*Fig 1* Low power magnification of nasal biopsy from patient with atrophic rhinitis The surface of the mucous membrane is covered by irregular scales (×42)

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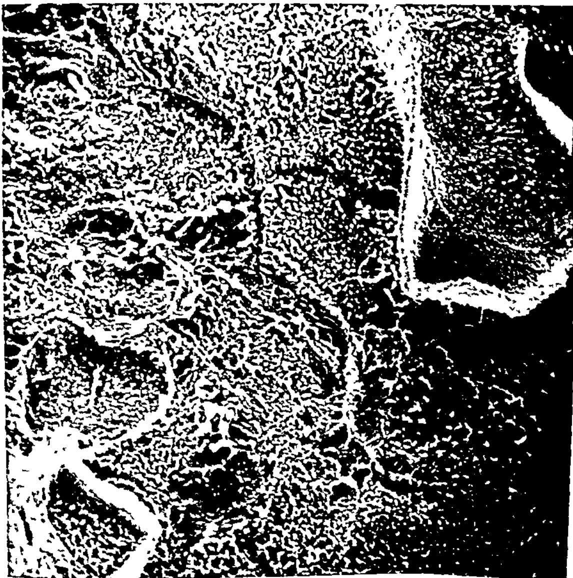


*Fig 2* Surface of the atrophic mucous membrane Medium power magnification In the upper right corner is seen a horny cell with elevation of its peripheral part, i e a normal desquamation process The surface structures of the cells are scattered short microvillous like projections ( 1 800)

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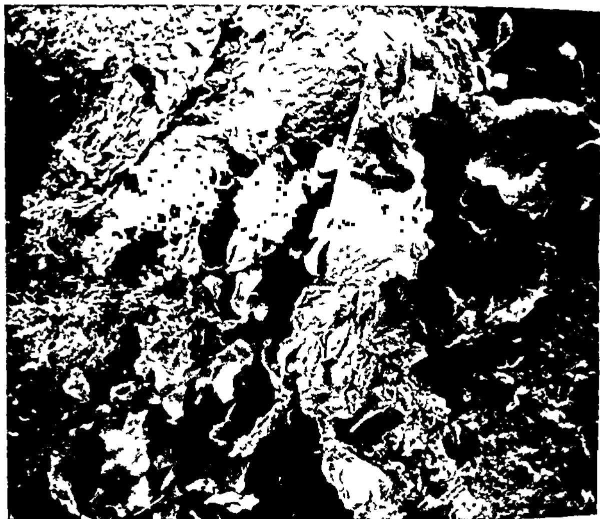
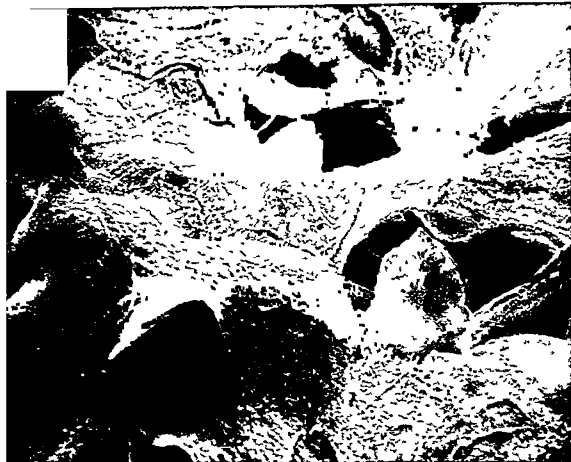


*Fig 3* Abnormal desquamation of horny cells forming large scales ( × 240)

epithelial type, i e a non keratinized epithelium without cilia and actual microvilli In these areas a single goblet cell was seen with its highly abnormal secretion being expelled from the cell but still in contact with the empty hole (Fig 6) The secretion looks dehydrated and viscid Apart from this the surface was not covered by secretion The polyp-like cytoplasma protuberances from the epithelial cells often found in normal nasal mucosa (Mygind & Bretlau 1974), are seldom observed in the atrophic specimens The same is the case with the openings from submucosal glands

## DISCUSSION

As shown earlier (Mygind & Bretlau 1973) it is possible to use biopsies for S E M studies if they are treated with extreme caution This offers new possibilities when examining the surface topography of different parts of the human respiratory tract under normal conditions as well as in respiratory tract diseases However the technique for the preparation of biological specimens for S E M is still under development and caution in interpreting the results is necessary The methods used for preparation of the specimens in the present study seem suitable as they produced results comparable to the pre



*ig 4* Abnormal desquamation of horny cells forming rregular dome like shapes ( × 1 000)

ure seen with the transmission electron microscope However, artefacts are inevitable in S E M For example the microvillous like projections may appear somewhat enlarged, because of the gold coating as the thickness of the gold layer may be of the same size as the projections themselves

Extensive light-microscopic studies of atrophic rhinitis have been carried out by Holopainen (1967) who also observed keratinized squamous epithelium in some biopsies from different locations in the nasal cavity In addition he showed that the squamous epithelium does not cover the total nasal cavity, but appears in islands This is in accordance with our results The occurrence of a horny squamous epithelium is very characteristic for atrophic rhinitis as it was found in only 4 out of 100 biopsies from the nasal cavity in patients with perennial rhinitis and in normal persons (unpublished data)

The surface-structures in atrophic rhinitis i e the microvillous like projections, are shorter and more scattered than the microvilli and cilia in the normal mucosa This decrease in the surface area of the atrophic mucosa renders it less capable of holding moisture, resulting in a severely impaired ability to resist dehydration When the epithelial surface is dry, the secretion from the scattered goblet cells is dehydrated *in situ*, as it is not transported away because of the lack of cilia The same is the case with the desquamated cells, which as large scales form the basis for the well known crusts Therefore, it seems that the primary feature in the crust formation



*Fig 3* Abnormal desquamation of horny cells forming large scales ( 240)

epithelial type i e a non keratinized epithelium without cilia and actual microvilli In these areas a single goblet cell was seen with its highly abnormal secretion being expelled from the cell but still in contact with the empty hole (Fig 6) The secretion looks dehydrated and viscid Apart from this the surface was not covered by secretion The polyp-like cytoplasma protuberances from the epithelial cells often found in normal nasal mucosa (Mygind & Bretlau 1974) are seldom observed in the atrophic specimens The same is the case with the openings from submucosal glands

## DISCUSSION

As shown earlier (Mygind & Bretlau 1973) it is possible to use biopsies for S E M studies if they are treated with extreme caution This offers new possibilities when examining the surface topography of different parts of the human respiratory tract under normal conditions as well as in respiratory tract diseases However the technique for the preparation of biological specimens for S E M is still under development and caution in interpreting the results is necessary The methods used for preparation of the specimens in the present study seem suitable as they produced results comparable to the p



*Fig 4* Abnormal desquamation of horny cells forming irregular dome like shapes (×1 000)

ture seen with the transmission electron microscope However artefacts are inevitable in S E M For example the microvillous like projections may appear somewhat enlarged because of the gold coating as the thickness of the gold layer may be of the same size as the projections themselves

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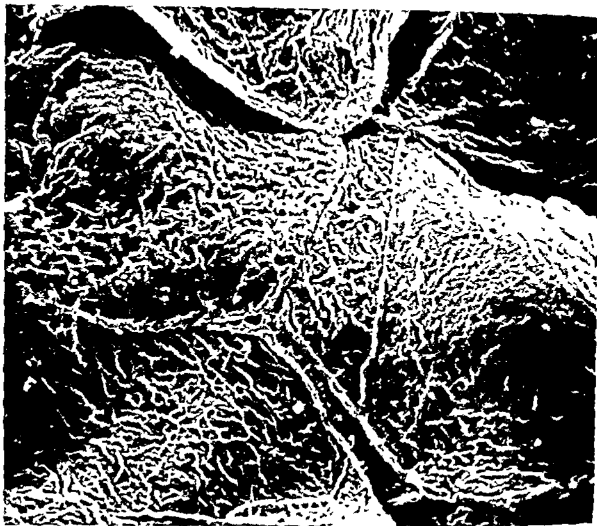


*Fig 5* Surface of the skin in the anterior part of the nasal cavity, in a normal person The surface structures are long branching folds (*arrow*) (× 2 330)

is a pathological pronounced desquamation and not an accumulation of neutrophilic leucocytes The desquamation may be so intense that the surface resembles eczematous skin

The similarities between the surface of the normal keratinized skin in the anterior part of the nasal cavity and the keratinized squamous epithelium of the metaplasized mucous membrane further back in the nose in atrophic rhinitis were striking There were only minor differences between the shape and surface of the cells, but the horny cells in the atrophic mucosa desquamated in an intense and pathological way, not observed in the normal skin

There was no consistent differences between the surface-structures on the top side and on the under side of the horny cells This means that no proliferation of microvillous like surface structures takes place from the free cell-surface The opposite is the case with the normal pseudostratified collumnar epithelium, in which microvilli as well as cilia proliferate at the surface of the cells

S E M studies of biopsies from normal persons and from patients with perennial rhinitis showed only minor differences (Mygind & Bretlau, 1973, 1974) It was impossible to decide whether a single biopsy originated from a normal

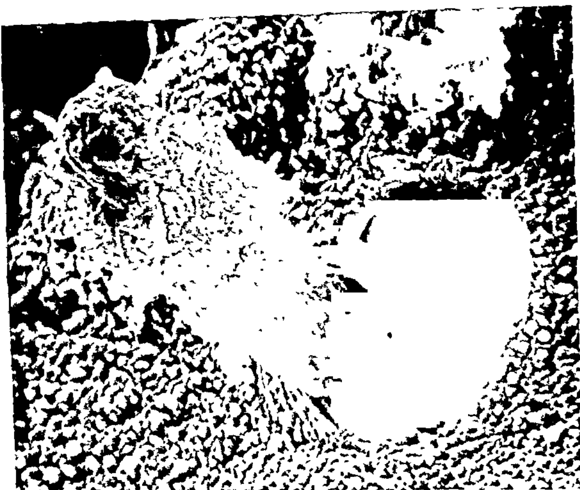


*Fig 6* Expelled mucus from a goblet cell Due to the lack of cilia, the mucus is not carried away and becomes dehydrated and viscid (×4 900)

person or from a patient with perennial rhinitis, whereas in the case of atrophic rhinitis, nearly all biopsies appeared highly abnormal

## ACKNOWLEDGMENT

The [illegible]

## ZUZAMMENFASSUNG

Biopsien der Nasenschleimhaut von Patienten mit atrophischer Rhinitis sind im Rasterelektronenmikroskop untersucht worden Es werden in weit verbreiteten Bereichen hautähnliche Plattenepithele festgestellt Der Umfang von *abgestossenen Schuppen, aus verhornten Zellen* bestehend, ist ausgeprägt und abnorm, und dieselben bilden die Basis fur die bekannten Schorfe Es sind nur sehr wenige Ausmundungen von submukosen Drusen und von Becherzellen vorhanden Die kleine Mukus-Menge trocknet in situ aufgrund von Mangel an Zilien aus

## REFERENCES

Holopainen, E 1967 Nasal mucous membrane in atrophic rhinitis with reference to symptomfree nasal mucosa *Acta Otolaryng* (Stockh), Suppl *227* Thesis

Lenz, H 1972*a* Die raumliche Darstellung von Zilien der menschlichen Nasenschleimhaut mit Hilfe des Rasterelektronmikroskops *Z Laryng Rhinol Otol 51*, 618

Table III *HSP 525 II Acute rhinitis*

| Patient number | 21 | 22 | 23 | 24 | 25 | 26 | 27 | 28 | 29 | 30 | 31 | 32 | 33 | 34 | 35 | 36 | 37 | 38 | 39 | 40 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Judgement of rhinomanometric recording | + | + | − | + | − | − | + | − | + | − | + | + | − | − | − | − | + | − | − | + |
| Placebo | | | × | | | × | | | | | | | × | | × | | | × | | |
| HS 592 | | | | × | × | | | | | | | × | | × | | | | | × | |
| HSP 525 II | | × | | | | | × | | × | | × | | | | | | | | | × |
| PPA | × | | | | | | | × | | × | | | | | | × | × | | | |

brought to coincide more closely with that of clemastine

The final series of tests on acute rhinitis consisted of 20 subjects The results are shown in Table IV, where HS 592 stands for clemastine 1 0 mg, PPA for phenyl propanolamine 50 mg retarded, and HSP 525 A for 50 mg PPA retarded with clemastine 1 0 mg There was no placebo effect and no effect of antihistamine alone The tests with both the combined preparation and the retarded phenylpropanolamine *were all positive The decongestive effect of the* combined drug and of the adrenergic substance on nasal blocking due to acute rhinitis is unequivocal Concerning the idea of synergism, the above-mentioned variation in patency in the subjects with rhinitis (see Fig 1) and the small number of subjects in this series do not permit antitative comparisons between HSP 525 A d PPA retarded However, there is no evidence *gainst the hypothesis of synergism*

*Concerning the histamine experiments,* the assessment of each individual experiment is explained in Fig 2 Three series were run, largely following the pattern of the experiments on acute rhinitis

In the first series 10 subjects were tested with placebo and with HSP 525 I (clemastine 1 0 mg with 50 mg phenylpropanolamine) There was no placebo effect, and the active drug was positive in all 5 cases

In a second series of 20 subjects HS 592 (clemastine 1 0 mg) PPA (30 mg phenylpropanolamine), and HSP 525 II were tested There was no placebo effect The antihistamine alone *was positive in one out of 5,* PPA positive in [illegible] out of 5, and HSP 525 II was positive in 3 out of 5 subjects

In the last series of experiments on another 20 test persons the phenylpropanolamine used was '50 mg retarded', HS 592 and HSP 525 A as in Table III above Again, as throughout the whole investigation, there was no placebo effect With the antihistamine alone one test out of 5 *was positive, with phenylpropanolamine retarded 4 out of 5 were positive,* and for the antihistamine combined with the retarded phenylpropanolamine all five were positive

Table IV *HSP 525 A Acute rhinitis*

| Patient number | 21 | 22 | 23 | 24 | 25 | 26 | 27 | 28 | 29 | 30 | 31 | 32 | 33 | 34 | 35 | 36 | 37 | 38 | 39 | 40 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Judgement of rhinomanometric recording | | + | + | | | + | + | − | − | + | | + | − | − | + | + | + | + | − | − |
| *Placebo* | | | | | | | | × | | | | | × | | | | | | | × |
| HS 592 | | | | | | | | | | | × | | | × | | | | | × | |
| HSP 525 A | | | | | | | × | | | × | | | | | | × | | × | | |
| PPA | | | | | | | | | | | | × | | | × | | × | | | |

The histamine series thus supported the hypothesis of synergism between antihistamine and adrenergic substance with regard to nasal decongestion, even with this type of experimental nasal blocking in man As can be seen from the typical experiment illustrated in Fig 2, the active drug seemed to be effective for at least $5^1/_2$ hours after administration

## DISCUSSION

In clinical praxis nose drops are used for decongestion of the nasal mucous membranes no matter what the cause of the congestion Topical administration of drugs has some drawbacks, however Nose drops used for more than one week often cause remote reactions, and the patient becomes 'hooked' on his nose drops This situation has not yet been reported in connexion with oral medication Further, with topical administration, drugs producing maximum therapeutical responses on the nano- or microgram blood level must be given in concentrations as high as 5 to 10 mg/ml As both nose drops and drugs given by mouth to produce nasal decongestion will act via the nasal blood vessels it would seem preferable to use the oral route, especially for long-term treatment In clinical praxis it is as a rule desirable also to achieve decongestion of adjoining mucous membranes derived from the upper airways—the mucosa within the sinuses and their ostia, the Eustachian tube, and the middle ear The chances of reaching these structures must be larger if the drug is distributed via the blood stream than if it is administered locally This is particularly important when blocking is due to inflammatory causes

Clinical testing in man has several shortcomings The subjects with nasal blocking due to acute rhinitis were very carefully selected, and represent only one type of inflammatory nasal blocking The variations in degree of nasal blocking before drug administration represent another source of error however, with the complete absence of placebo effects the results must be regarded as conclusive The drug combination marked 5031 had a decongestive effect in 10 out of 15 tests The combination clemastine with phenylpropanolamine showed still better results, with 14 positive tests of 15, disregarding some galenic differences in the composition of the drug tested (HSP 525 I, HSP 525 II, and HSP 525 A) The significance of the results is proved by the thirty placebo tests, all of which were negative The objective rhinomanometric technique used here shows that oral administration is the method of choice for obtaining nasal decongestion even when the nasal obstruction is due to inflammation

A series of studies by Jackson (1971), Bentley & Jackson (1970), and Hall & Jackson (1968), on nasal patency, the sinuses, and the Eustachian tube in dogs, indicate that the mucous membranes in the different organs behave rather similarly from a pharmacological point of view From their experiments it is evident that the nasal blood flow is regulated via nervous and pharmacological pathways Dahlström & Fuxe (1965) have demonstrated the adrenergic innervation of the nasal blood vessels They have also shown that no adrenergic fibres are present round the glands of the nasal mucosa in their animals, an interesting fact often neglected in connexion with nasal patency Cauna & Hinderer (1969) have demonstrated by electron microscopy a dense innervation of the blood vessels in the human nasal mucosa All the investigations quoted are of interest because they in different ways indicate that the decongestion obtained in the nasal mucosa by oral medication also occurs in adjoining structures, thus extending the indications for the use of such drugs

The synergism between antihistamine and adrenergic substances in allergic nasal congestion is documented (McLaurin et al (1961), Pullen & Montgomery (1963), Aschan (1964), and others) Bentley & Jackson (1970) in experiments on dogs found a synergistic effect of several anti histamines and epinephrine upon histamine-provoked nasal blocking The results of the experiments on acute rhinitis and of the series with histamine-provoked nasal congestion in man support the idea The reason for studying

Table III *HSP 525 II Acute rhinitis*

| Patient number | 21 | 22 | 23 | 24 | 25 | 26 | 27 | 28 | 29 | 30 | 31 | 32 | 33 | 34 | 35 | 36 | 37 | 38 | 39 | 40 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Judgement of rhinomanometric recording | + | + | − | + | − | − | + | − | + | − | + | + | | − | | − | + | − | − | + |
| Placebo | | | × | | | × | | | | | | | × | | × | | | × | | |
| HS 592 | | | | × | × | | | | | | | × | | × | | | | | × | |
| HSP 525 II | | × | | | | | × | | × | | × | | | | | | | | | × |
| PPA | × | | | | | | | × | | × | | | | | | × | × | | | |

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The final series of tests on acute rhinitis consisted of 20 subjects The results are shown in Table IV, where HS 592 stands for clemastine 1 0 mg PPA for phenyl-propanolamine 50 mg retarded, and HSP 525 A for 50 mg PPA retarded with clemastine 1 0 mg There was no placebo effect and no effect of antihistamine alone The tests with both the combined preparation and the retarded phenylpropanolamine were all positive The decongestive effect of the combined drug and of the adrenergic substance on nasal blocking due to acute rhinitis is unequivocal Concerning the idea of synergism, the above-mentioned variation in patency in the subjects with rhinitis (see Fig 1) and the small number of subjects in this series do not permit quantitative comparisons between HSP 525 A and PPA retarded However, there is no evidence against the hypothesis of synergism

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|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Judgement of rhinomanometric recording | | + | + | − | − | + | + | − | − | + | − | + | − | | + | + | + | + | − | − |
| Placebo | | | | | | | | × | × | | | | × | | | | | | | × |
| HS 592 | | | | | × | | | | | | × | | | × | | | | | × | |
| HSP 525 A | | | | | | | × | | | × | | | | | | × | | × | | |
| PPA | | | × | | | × | | | | | | × | | | × | | × | | | |

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clemastine in greater detail in this investigation was that it is known to produce little or no sedative effect Phenylpropanolamine was chosen because of certain undesirable side-effects of ephedrine, particularly in elderly subjects

The value of rhinomanometry in investigations of this type must be strongly emphasized The reproducibility of the individual controls in the *histamine provocation shown in Fig 2 is one* way of illustrating this The results of Büsser & Schibli (1973), who used a somewhat different technique, provide further confirmation (see also Bühlmann, 1973) The complete absence of placebo effect in the double-blind series is further proof that rhinomanometry is the method of choice in clinical trials of this type

## ZUSAMMENFASSUNG

Klinische Versuche mit Antihistaminika in Kombination mit adrenergischen Substanzen ergaben einen dekongestiven Effekt bei Schwellung der Nasenschleimhaut aufgrund akuter Rhinitis Doppelblindtechnik verbunden mit objektiver Rhinomanometrie uber einen Zeitraum von 9 Stunden bei jedem einzelnen Versuch zeigte das vollstandige Ausbleiben des Placebo-effekts die Resultate erwiesen sich daher als unverlässig Versuche, durchgefuhrt an 85 Patienten mit akuter Rhinitis, sowie Versuche mit 50 normalen Versuchspersonen mit histamin-provozierter Blockade der Nase unterstutzten die Annahme, dass Antihistamin zusammen mit einer adrenergischen Substanz Synergismus mit Wirkung auf die nasale Dekongestion zeigt Von den untersuchten Praparaten erzielte man die besten Ergebnisse mit einer Kombination von Clemastine 1 0 mg und Phenylpropanolamine 50 mg

## REFERENCES

Aschan G 1964 Experimentell klinisk provning av H hydroxiaetyl promethazin, chlorid *(Aprobit®) kombinerat* med ephedrin hydrochlor pa naspassagen. *Svensk Lakartidn 61*, 3125

Aschan, G, Drettner, B & Ronge, H 1956 En objektiv metod for matning och registrering av näsandnings motståndet *Svensk Otolaryng For Forh* Hafte 2

— 1958 A new technique for measuring nasal resistance to breathing illustrated by the effects of histamine *and physical effort Ann Acad Sci Upsaliensis 2, 111*

Bentley, A J & Jackson, R T 1970 Changes in the patency of the upper nasal passage induced by histamine and antihistamines *Laryngoscope 80* 1859

Bühlmann, A A 1973 Measure of the breathing resistance in the nasal passage *Trans X Int World Congr Otorhinolaryngol* Excerpta Medica To be published

Büsser, E & Schibli, R A 1973 Rhinomanometrie Methodik und Normalwerte *Deutsch Med Wschr 98* 719

Cauna N 1970 Electron microscopy of the nasal vascular bed and its nerve supply *Ann Otol 79*, 443

Cauna, N & Hinderer, K H 1969 Fine structure of blood vessels of the human nasal respiratory mucosa. *Ann Otol 78*, 865

Dahlstrom, A & Fuxe, K 1965 The adrenergic innervation of the nasal mucosa of certain mammals *Acta Otolaryng* (Stockh) 59, 65

Hall L J & Jackson, R T 1968 Effects of alpha and beta adrenergic agonists on nasal blood flow *Ann Otol 77*, 1120

Jackson R T 1971 Pharmacological mechanisms in the Eustachian tube *Ann Otol 80*, 313

McLaurin, J W, Shipman, W F & Rosedale Jr, R *1961 Oral decongestants A double blind comparison* study of the effectiveness of four sympathomimetic drugs, objective and subjective *Laryngoscope 71 54*

Pullen II, F W & Montgomery, W W 1963 Comparative evaluation of oral decongestants *Arch Otolaryng* (Chic) 77, 10

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# ULTRASTRUCTURE OF THE EPITHELIUM IN ATROPHIC RHINITIS

## *Scanning Electron Microscopic Studies*

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(Received October 29, 1973)

*Abstract* Biopsies from nasal mucosa of patients with atrophic rhinitis were studied in the scanning electron microscope A keratinized squamous epithelium, resembling skin is seen over large areas Desquamation of horny cells is pronounced and abnormal, with large desquamated scales forming the basis for the well known crusts Openings from submucosal glands and goblet cells are very infrequent The small amount of mucus is dehydrated in situ, because of the lack of cilia

The great depth of focus of scanning electron microscopy (SEM), its wide range of magnifications, and relatively great resolving power, make it a logical tool for the observation of the delicate structures of the surface of the human nasal mucosa Within the last few years, several papers have appeared dealing with S E M studies of the normal nasal mucosa and the nasal mucosa from patients with perennial rhinitis (Lenz, 1972*a*, *b*, 1973, Mygind & Bretlau, 1973, 1974) In addition Lenz (1972*c*) studied the squamous epithelium in the anterior part of the nasal cavity and described the normal desquamation process of the epithelial cells In the present study the fine-structures of the horny cells and their spatial relationship within the squamous epithelium in atrophic rhinitis have been investigated An attempt has been made to correlate these observations with information available from transmission electron microscopic investigations (Mygind et al , 1974)

## MATERIAL AND METHODS

Six adult patients with pronounced atrophic rhinitis were examined For a longer period of time they had all suffered from crust formation in the nose and they had only been treated by conservative means Biopsies of a size of 1½–3 mm were taken with a small forceps without local anaesthesia They were taken from the lower edge of the inferior turbinate ½–1 cm behind the anterior edge as well as from other places in the nasal cavity

For comparison purposes, biopsies were also obtained from the anterior part of the nasal cavity, at the borderline to the skin, in 3 normal persons

As glutaraldehyde causes epithelium to adhere to the forceps, the biopsies were loosened from the forceps in 0 9 % sodium cloride and immediately thereafter transferred to a cold 5 % solution of glutaraldehyde, buffered with a 0 03 M sodium cacodylate (pH 7 4) After 24 hours fixation the biopsies were stored in the buffer After dehydration in ethyl alcohol the specimens were transferred to benzene After freeze-drying as described by Nørrevang & Wingstrand (1970) the specimens were mounted on stubs and a conduction coat of gold was deposited in vacuum The specimens were studied in a Cambridge Stereoscan S2

clemastine in greater detail in this investigation was that it is known to produce little or no sedative effect Phenylpropanolamine was chosen because of certain undesirable side-effects of ephedrine, particularly in elderly subjects

The value of rhinomanometry in investigations of this type must be strongly emphasized The reproducibility of the individual controls in the histamine provocation shown in Fig 2 is one way of illustrating this The results of Büsser & Schibli (1973), who used a somewhat different technique, provide further confirmation (see also Bühlmann, 1973) The complete absence of placebo effect in the double-blind series is further proof that rhinomanometry is the method of choice in clinical trials of this type

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bination von Clemastine 1,0 mg und Phenylpropanolamine 50 mg

## REFERENCES

Aschan, G 1964 Experimentell klinisk *provning av H hydroxiaetyl promethazin, chlorid* (Aprobit®) kombinerat med ephedrin hydrochlor på näspassagen *Svensk Lakartidn 61*, 3125

Aschan G Drettner, B & Ronge, H 1956 *En objektiv metod for mätning och registrering av näsandnings* motståndet *Svensk Otolaryng For Forh* Häfte 2

— 1958 A new technique for measuring nasal resistance to breathing illustrated by the effects of histamine and physical effort *Ann Acad Sci Upsaliensis 2* 111

Bentley, A J & Jackson, R T 1970 Changes in the [illegible] *Otorhinolaryngol* Excerpta Medica To be published

*Busser, E & Schibli, R A 1973 Rhinomanometrie Me*thodik und Normalwerte *Deutsch Med Wschr 98* 719

Cauna, N 1970 Electron microscopy of the nasal vascular bed and its nerve supply *Ann Otol 79* 443

Cauna N & *Hinderer, K H 1969* Fine structure of blood vessels of the human nasal respiratory mucosa *Ann Otol 78*, 865

Dahlstrom, A & Fuxe, K 1965 The adrenergic innervation of the nasal mucosa of certain mammals *Acta Otolaryng* (Stockh ) *59*, 65

Hall, L J & Jackson, R T 1968 Effects of alpha and *beta adrenergic agonists on nasal blood flow Ann Otol 77*, 1120

Jackson R T 1971 Pharmacological mechanisms in the Eustachian tube *Ann Otol 80*, 313

McLaurin, *J W*, Shipman, *W F & Rosedale Jr*, R 1961 Oral decongestants A double blind comparison study of the effectiveness of four sympathomimetic drugs, objective and subjective *Laryngoscope* 71 54

Pullen II F W & Montgomery, W W 1963 Comparative evaluation of oral decongestants *Arch Otolaryng* (*Chic* ) *77*, 10

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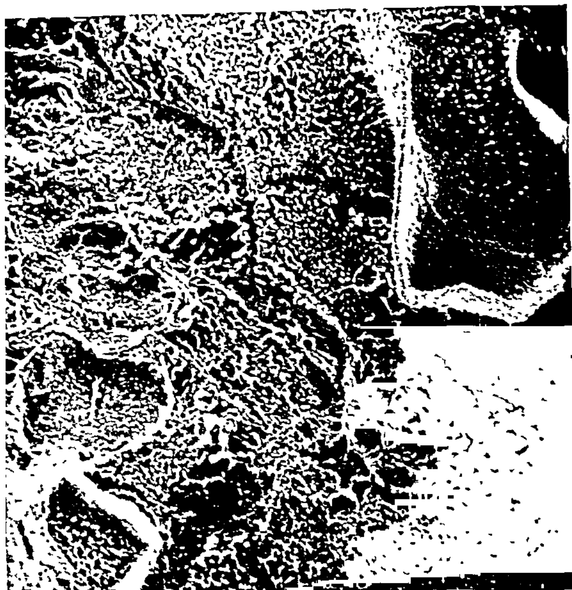


*Fig 2* Surface of the atrophic mucous membrane Medium-power magnification In the upper right corner is seen a horny cell with elevation of its peripheral part, i e a normal desquamation process. The surface-structures of the cells are scattered, short, microvillous like projections ( × 1 800)

(Fig 2) In contrast to the horny cells in the normal skin, where long, branching folds are the dominant surface structure, the cell surfaces of the atrophic mucosa are mostly covered by short microvillous-like projections (Fig 2) These projections *may be remnants of desmosomes. On* the whole, the surface structures of the atrophic mucosa are much shorter and more scattered than in the normal mucosa (Lenz, 1972*a*, Mygind & Bretlau, 1973) Actual microvilli (1–2 $\mu$m long structures) are not seen in the squamous epithelium

In the specimens investigated from patients with atrophic rhinitis the dominant type of epithelium was a *horny squamous epithelium*, found in 4 out of 6 patients In one biopsy, normally looking cilia were seen. In nearly half of the biopsies, areas were observed of an intermediate



*Fig 1* Low power magnification of nasal biopsy from patient with atrophic rhinitis The surface of the mucous membrane is covered by irregular scales ( × 42)

## RESULTS

The surface-topography of the epithelium is revealed in considerable detail in the scanning microscope, which also gives a striking three dimentional appearance At low power magnification the atrophic mucosa looks highly ab normal, with the appearance of "reptile skin" with numerous scales, irregularly arranged and of unequal size (Fig 1) At higher magnification one can see horny cells desquamate in the same manner as the horny cells in the anterior part of the nasal cavity, i e by initial elevation of the peripheral part of the cell (Fig 2) However, in the atrophic mucosa it is much more common to see several cells loosen *in toto*, forming large scales (Fig 3) or irregular dome-like shapes (Fig 4) This suggests that the cells are quite flexible Even though the horny cells are extremely thin, no examples of torn or fragmented cells were seen

The horny cells in the skin of the anterior part of the normal nasal cavity are polygonal or hexagonal in shape and have smooth straight intercellular junctions (Fig 5) One frequently encounters the junction of three cells at the corners of the contiguous hexagons The cell surface is not smooth, but covered by long, branching folds

Whereas the picture of the normal nasal mucosa is quite different from this (Mygind & Bretlau, 1973, 1974) the atrophic nasal mucosa even in the posterior part of the nose has several similarities to the normal skin only minor differences are seen The horny cells in the atrophic mucosa may be polygonal in shape as the cells in the skin, but they are often more roundish and it may be difficult to discern the cell borders



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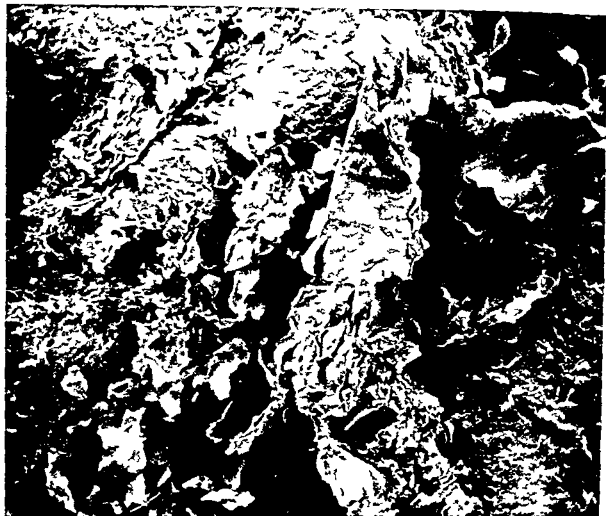


Fig 3 Abnormal desquamation of horny cells forming large scales ( 240)

epithelial type i e a non keratinized epithelium without cilia and actual microvilli In these areas a single goblet cell was seen with its highly abnormal secretion being expelled from the cell but still in contact with the empty hole (Fig 6) The secretion looks dehydrated and viscid Apart from this the surface was not covered by secretion The polyp like cytoplasma protuberances from the epithelial cells often found in normal nasal mucosa (Mygind & Bretlau 1974) are seldom observed in the atrophic specimens The same is the case with the openings from submucosal glands

## DISCUSSION

As shown earlier (Mygind & Bretlau 1973) it is possible to use biopsies for S E M studies if they are treated with extreme caution This offers new possibilities when examining the surface topography of different parts of the human respiratory tract under normal conditions as well as in respiratory tract diseases However the technique for the preparation of biological specimens for S E M is still under development and caution in interpreting the results is necessary The methods used for preparation of the specimens in the present study seem suitable as they produced results comparable to the p c



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The surface structures in atrophic rhinitis, i e the microvillous like projections, are shorter and more scattered than the microvilli and cilia in the normal mucosa This decrease in the surface area of the atrophic mucosa renders it less capable of holding moisture, resulting in a severely impaired ability to resist dehydration When the epithelial surface is dry, the secretion from the scattered goblet cells is dehydrated *in situ*, as it is not transported away because of the lack of cilia The same is the case with the desquamated cells, which as large scales form the basis for the well-known crusts Therefore, it seems that the primary feature in the crust formation

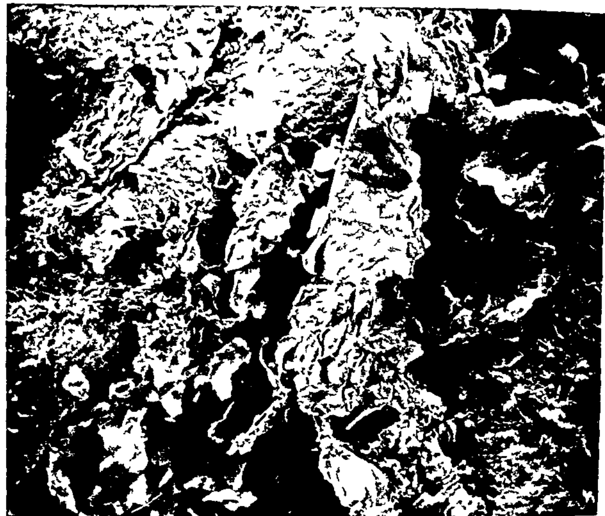


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Extensive light-microscopic studies of atrophic hinitis have been carried out by Holopaiien (1967) who also observed keratinized squamous epithelium in some biopsies from different ocations in the nasal cavity In addition he showed that the squamous epithelium does not cover the total nasal cavity, but appears in islands This is in accordance with our results The occurrence of a horny squamous epithelium is very characteristic for atrophic rhinitis, as it was found in only 4 out of 100 biopsies from the nasal cavity in patients with perennial rhinitis and in normal persons (unpublished data)

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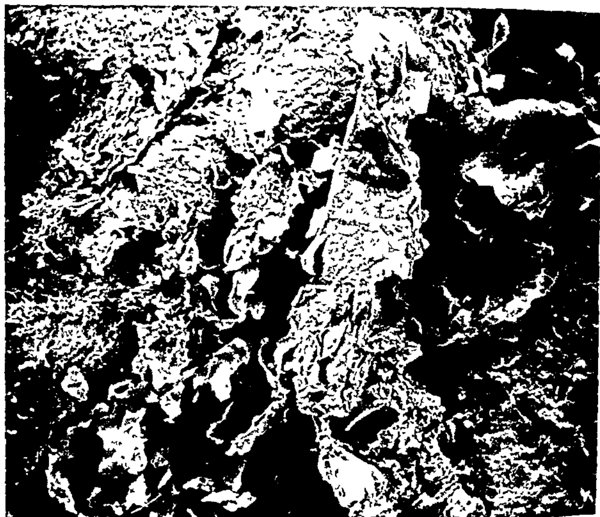


*Fig. 3.* Abnormal desquamation of horny cells forming large scales (×240).

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## DISCUSSION

As shown earlier (Mygind & Bretlau, 1973), it is possible to use biopsies for S.E.M. studies, if they are treated with extreme caution. This offers new possibilities when examining the surface topography of different parts of the human respiratory tract under normal conditions, as well as in respiratory tract diseases. However, the technique for the preparation of biological specimens for S.E.M. is still under development, and caution in interpreting the results is necessary. The methods used for preparation of the specimens in the present study seem suitable, as they produced results comparable to the pic-



Fig 4 Abnormal desquamation of horny cells forming irregular, dome like shapes (× 1 000)

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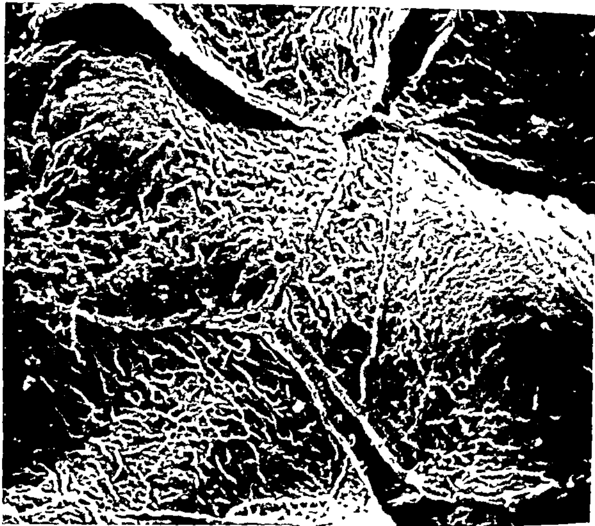


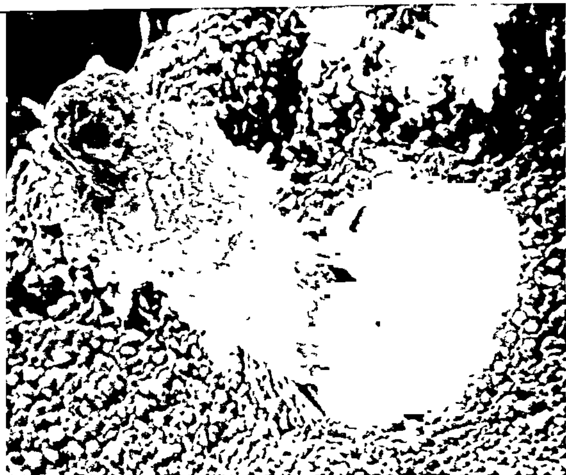
*Fig 5* Surface of the skin in the anterior part of the nasal cavity in a normal person The surface structures are long branching folds (*arrow*) ( × 2 330)

is a pathological pronounced desquamation and not an accumulation of neutrophilic leucocytes The desquamation may be so intense that the surface resembles eczematous skin

The similarities between the surface of the normal keratinized skin in the anterior part of the nasal cavity and the keratinized squamous epithelium of the metaplasized mucous membrane further back in the nose in atrophic rhinitis were striking There were only minor differences between the shape and surface of the cells but the horny cells in the atrophic mucosa desquamated in an intense and pathological way, not observed in the normal skin

There was no consistent differences between the surface structures on the top side and on the under side of the horny cells This means that no proliferation of microvillous like surface-structures takes place from the free cell surface The opposite is the case with the normal pseudostratified collumnar epithelium in which microvilli as well as cilia proliferate at the surface of the cells

S E M studies of biopsies from normal persons and from patients with perennial rhinitis showed only minor differences (Mygind & Bretlau, 1973 1974) It was impossible to decide whether a single biopsy originated from a normal



g *6* Expelled mucus from a goblet cell Due to the ck of cilia, the mucus is not carried away and becomes hydrated and viscid (× 4 900)

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## ACKNOWLEDGMENT

he Cambridge Stereoscan microscope was kindly placed

en and Mr Erik Jensenius for skilful technical assistance

## ZUZAMMENFASSUNG

Biopsien der Nasenschleimhaut von Patienten mit atrophischer Rhinitis sind im Rasterelektronenmikroskop untersucht worden Es werden in weit verbreiteten Bereichen hautahnliche Plattenepithele festgestellt Der Umfang von abgestossenen Schuppen, aus verhornten Zellen bestehend, ist ausgeprägt und abnorm, und dieselben bilden die Basis fur die bekannten Schorfe Es sind nur sehr wenige Ausmundungen von submukosen Drusen und von Becherzellen vorhanden. Die kleine Mukus-Menge trocknet in situ aufgrund von Mangel an Zilien aus

## REFERENCES

Holopainen E. 1967 Nasal mucous membrane in atrophic rhinitis with reference to symptomfree nasal mucosa. *Acta Otolaryng* (Stockh.), Suppl *227* Thesis

Lenz, H 1972*a* Die räumliche Darstellung von Zilien der menschlichen Nasenschleimhaut mit Hilfe des Rasterelektronmikroskops *Z Laryng Rhinol Otol 51,* 618

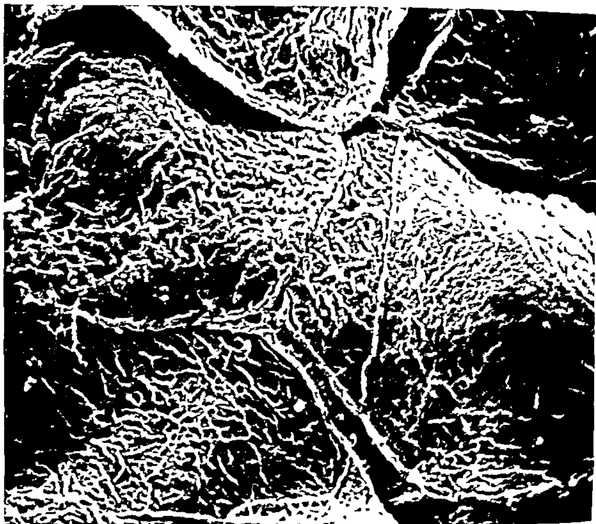


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— 1972*b* Die Oberfläche der Nasenschleimhaut bei Rhinitis vasomotorica im Rasterelektronmikroskop *Arch Klin Exp Ohr Nas Kehlkopfheilk 202*, 353
— 1972*c* Der Abschliferungsvorgang am Plattenepithel der Nasenschleimhaut des Menschen Eine rasterelektronmikroskopische Studie *Arch Klin Exp Ohr Nas Kehlkopfheilk 203*, 30
— 1973 Three-dimensional surface representation of the cilia free nasal mucosa of man *Acta Otolaryng* (Stockh) *76*, 47
Mygind N & Bretlau, P 1973 Scanning electron microscopic studies of the human nasal mucosa in normal persons and in patients with perennial rhinitis I Cilia and microvilli *Acta Allerg* (Kbh) *28*, 9
— 1974 Op cit II Secretion *Acta Allerg* (Kbh) In press
Mygind, N, Thomsen, J & Balslev Jorgensen, M 1974 Ultrastructure of the epithelium in atrophic rhinitis. Transmission electron microscopic studies *Acta Otolaryng* (Stockh) In press
Nørrevang, A & Wingstrand, K G 1970 On the occurrence and structure of choanocyte like cells in some echinoderms *Acta Zool* (Stockh) *51*, 249

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